

HORMONES
A SURVEY OF THEIR PROPERTIES
AND USES

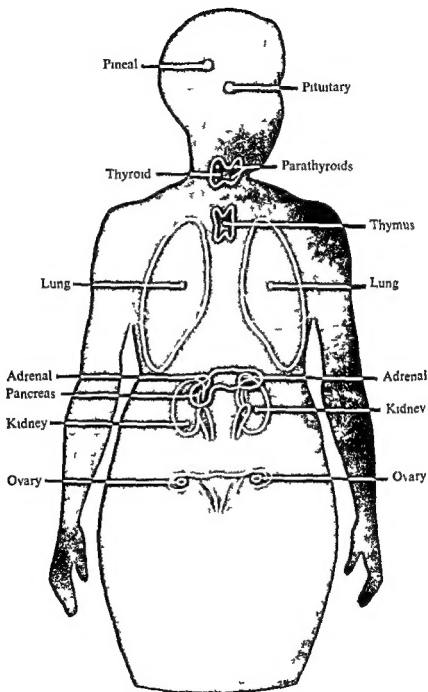


FIG. 1

POSITION OF ENDOCRINE GLANDS IN THE FEMALE

The pineal and thymus glands are included in the diagram although their functions are still obscure. The lungs and kidneys are shown to indicate their position relative to the glands.

HORMONES:

A SURVEY OF THEIR PROPERTIES AND USES

*Published by direction of the Council of
The Pharmaceutical Society of Great Britain*

L O N D O N

The Pharmaceutical Press 17 Bloomsbury Square W C 1

1951

PREFACE

The chemical messengers however or hormones (from ὁρμῶν I excite or arouse) as we might call them E H STARLING
MD FRCP FRS The Croonian Lectures 1905

In the forty six years since they were so named by Starling the number of substances now recognised as hormones has increased considerably and the available information is far too extensive to be included in one volume. In selecting the material for this book the aim has been to provide pharmacists, medical practitioners and students with an account of those hormones and endocrine glands which have well defined pharmacological effects and therapeutic applications together with information on closely related substances such as the artificial hormones and antihormones. It is for this reason that the pineal and thymus glands and a number of hormones including secretin have been omitted. It should be noted that the term 'artificial hormone' is preferred to 'synthetic hormone' for denoting those substances with hormonal activity which are produced solely in the laboratory since many naturally occurring hormones can now be completely synthesised. Many original papers are quoted in the text, references to which are given at the end of each chapter. These together with an extensive bibliography should be of value to laboratory workers and others who may wish to obtain further details of particular aspects of the subject.

Beginning with an historical account of the development of the science of endocrinology from early work with gland extracts to the isolation and synthesis of active principles, the book continues with an account of the physiology of the glands in which the normal function and the effects of dysfunction of each gland are described and the importance and limitations of replacement therapy outlined.

The chemistry of the hormones may conveniently be divided into two sections. Those hormones having a steroid nucleus form a group of closely related and well defined substances, whereas the non steroid hormones are chemically of a heterogeneous nature, having a variety of structures. Such an arrangement has been adopted in this book. In addition the chapter on the chemistry of the steroid hormones opens with a brief account of the general chemistry of steroids to serve as a basis for explaining the more detailed chemistry of the hormones themselves. It also includes an account of the artificial hormones since these are related chemically or physiologically to certain of the steroid hormones.

As more and more hormones are isolated and characterised, so biological methods of assay tend to be replaced by chemical methods.

Nevertheless there are still many hormones which are assayed biologically and in the chapter on standardisation there is a full account of the principles underlying the application of biological methods and descriptions of the more important assays together with an explanation of the various units which are or have been used to express activity

The chapter on action and uses deals with the pharmacology and therapeutics of the glands and their hormones and of the artificial hormones and antihormones. It indicates the various forms in which the substances are administered and also describes the method of administration by implantation. Preparations referred to in this chapter are described more fully in the next which deals with the pharmacy of hormones and related substances and their preparations. Methods and formulæ are given for a variety of preparations including injections, solutions, ointments, creams and suppositories. Methods of sterilisation, conditions for storage and the requirements of the Therapeutic Substances Act 1925 and Regulations made thereunder and of the Pharmacy and Poisons Act 1933 in so far as they apply to glands and hormones are also described. The final chapter gives details of the commercial forms of many of the preparations referred to in other parts of the book and the names and addresses of the manufacturers.

The abbreviations used for weights and measures are in accordance with those of the British Pharmacopœia 1948 and temperatures are expressed in degrees centigrade.

The Council of the Pharmaceutical Society is indebted to the following contributors for preparing the chapters indicated: H. E. Dale (Physiology and Action and Uses), C. W. Emmens, D.Sc., Ph.D. (Standardisation), D. H. Hey, D.Sc., Ph.D., F.R.I.C. (Chemistry) and T. D. Whittet, Ph.C., D.B.A. (Pharmacy). It is also indebted to P. M. F. Bishop, D.M., J. F. Wilkinson, M.D., F.R.C.P. and P. R. Evans, F.R.C.P., M.D. for clinical photographs and advice and to the chief pharmacists of many hospitals for certain pharmaceutical formulæ.

June
1951

CONTENTS

	Page
PREFACE	vii
INTRODUCTION	1
Chapter I HISTORY	5
Chapter II PHYSIOLOGY	
Thyroid	9
Hypothyroidism Hyperthyroidism Microscopical Characters Thyroid and Metamorphosis Thyroid and Other Endocrine Glands Basal Metabolic Rate Radioactive Iodine Iodinated Proteins Antithyroid Substances	
Parathyroids	15
Effects of Parathyroidectomy Hypoparathyroidism Hyperpara- thyroidism Calcium and Phosphorus Metabolism Parathyroid Hormone and Tetany Dihydroxycholesterol and Calciferol	
Pancreas	19
Carbohydrate Metabolism Action of Insulin Blood Sugar Levels Glucose Tolerance Test Insulin Preparations Biochemical Exami- nations Pancreas and Other Endocrine Glands Alloxan Diabetes Phloridzin Diabetes Lipocain	
Pituitary	
Anterior Lobe	28
Growth Hormone Anterior Lobe and the Gonads Chorionic Gonadotrophin Serum Gonadotrophin Anterior Lobe and Other Gland	
Posterior Lobe	33
Pars Intermedia	33
Adrenals	
Adrenal Medulla	34
Action of Adrenaline Action of No adrenaline	
Adrenal Cortex	37
Gonads	38
Male Reproductive System Female Reproductive System Repro- ductive Cycle in Mammals Human Reproductive or Menstrual Cycle	
Pregnancy Diagnosis	46
Aschheim Zondek Test Friedman Test Xenopus Test Male Toad Tests Cullman Test	
References	48
Chapter III CHEMISTRY OF THE NON-STEROID HORMONES	
Thyroid	50
Thyroxine	
Parathyroids	53
Pancreas	53
Insulin	

Pituitary	Page 55
Posterior Lobe Anterior Lobe	
Adrenals	56
Adrenal Medulla Adrenaline Noradrenaline	
References	59
Chapter IV CHEMISTRY OF THE STEROID HORMONES	
Sterols	61
Cholesterol Sitosterol Stigmasterol Ergosterol	
Bile Acids	64
Aglycones of the Cardiac Glycosides	66
Sapogenins	66
Stereochemistry	67
Sex Hormones	69
Oestrogens	71
Isolation Oestrone α Oestradiol Oestriol (+) Equilenin	
Equilin	
Androgens	74
Isolation Androsterone Dehydroandrosterone Testosterone	
Progesterone	76
Isolation	
Adrenal Cortical Hormones	77
Isolation Corticosterone Deoxycortone Dehydrocorticosterone	
Cortisone Adrenosterone	
Synthesis of the Steroid Hormones	79
Oestrone Oestradiol and Oestriol Equilenin Androsterone	
Dehydroandrosterone Testosterone Progesterone Cortisone	
Deoxycortone Dehydrocorticosterone Cortisone	
Artificial Hormones	99
Sulboestrol Hexoestrol Dienoestrol Triphenylethylene Deriva	
tives Steroid Derivatives Methyltestosterone Ethisterone	
Ethinylestradiol Douynol c Acid and Derivatives	
Excretion Products	106
References	108
Chapter V STANDARDISATION	
International Standards	111
Biological Assay	113
Quantal Responses Choosing a Test method Computat on	
Limits of Error	
Methods of Assaying Relative Potency	118
Anterior Lobe of the Pituitary	119
Thyrotrophic Hormone Lactogenic Hormone Growth Hormone	
Adrenocorticotrophic Hormone Gonadotrophins	
Chorionic Gonadotrophin	123
Serum Gonadotrophin	125
Posterior Lobe of the Pituitary	125
Oxytocic Act ity Ant diuretic Activity Pressor Activity	

Adrenals	127
Adrenal Cortical Hormones Adrenaline	
Thyroid	129
Xenopus Tadpole Method Enclosed Vessel Technique with Mice	
Parathyroids	130
Pancreas	
Insulin (Ordinary Insulin)	131
Mouse Convulsion Method Rabbit Blood Sugar Method	
Delayed Action Insulins	132
Gonads	132
Estrogens Progesterone Androgens	
Relative Accuracy of Test Methods	135
References	136
Chapter VI ACTION AND USES	
Thyroid	139
Hypothyroidism Hyperthyroidism Other Uses of Thyroid Thyroid Preparations	
Parathyroids	143
Hypoparathyroidism Dihydroxycholesterol in Tetany Calciferol in Tetany Hyperparathyroidism	
Pancreas	146
Action of Insulin Diabetes Mellitus Insulin Preparations Action of Different Insulin Preparations Dosage Vitamins and Blood Sugar Hypoglycemia Diabetic Coma Insulin in Non-diabetic Conditions	
Pituitary	
Anterior Lobe	151
Growth Hormone Lactogenic Hormone Adrenocorticotrophic Hormone Thyrotrophic Hormone Gonadotrophins	
Posterior Lobe	158
Adrenals	159
Adrenaline Cortical Extracts Deoxycortone Acetate Implan- tation Sublingual Administration Cutaneous Inunction Deoxy- cortone Acetate in Rheumatism Deoxycortone Acetate in Shock	
Gonads	163
Estrogens	164
Natural Estrogens Artificial Estrogens Action of Estrogens Methods of Administration Toxicity Clinical Applications Estrogens and Cancer	
Progestogens	169
Clinical Applications	
Androgens	170
Clinical Applications Use in Cancer of the Breast	
Allergy to Hormones	172
References	173
Chapter VII PHARMACY	
Preparations of Hormones of Unknown Constitution	176
Parathyroids	176
Parathyroid Injection	

Pancreas	176
Injectio Insulinae Injectio Insulinae Protaminati cum Zinco Injectio Insulinae Globuli cum Zinco Preservation of Insulin Identification of Insulin Packs Insulin Tablets Insulin for Local Application	
Anterior Lobe of the Pituitary	179
Growth Hormone Gonadotrophic Hormones Lactogenic Hormone Thyrotrophic Hormone Adrenocorticotrophic Hormone	
Pituitary like Gonadotrophins	180
Gonadotrophinum Chorionicum Gonadotrophinum Sericum Mixtures of Gonadotrophic Hormones	
Posterior Lobe of the Pituitary	181
Injectio Pituitarii Posterioris Injectio Oxytocini Injectio Vasopressini Pituitary (Posterior Lobe) Emulsion Pituitary (Posterior Lobe) Powder	
Preparations of Hormones of Known Constitution	183
Thyroid	183
Thyroxine Antithyroid Substances	
Adrenals	184
Adrenalina Liquor Adrenalinæ Hydrochloridi Injectio Adrenalinæ Other Adrenaline Preparations	
Steroid Hormones	186
Oral Administration Sublingual Administration Percutaneous Administration Vaginal and Rectal Administration Nasal Administration Parenteral Administration Implantation Hormone Allergy Test Sets	
Legal Requirements	188
References	190
 Chapter VIII — COMMERCIAL PREPARATIONS	192
 BIBLIOGRAPHY	207
 INDEX	212

ILLUSTRATIONS

Figure	Page
1 Position of Endocrine Glands in the Female	<i>Frontispiece</i>
2 Position of Thyroid	9
3 Sections of Thyroid	10
4 Induced Metamorphosis in Axolotl	11
5 South African Clawed Toad	12
6 Position of Parathyroids	16
7 Position of Pancreas	19
8 Section of Mammalian Pancreas	20
9 Glucose Tolerance Test	22
10 Insulin Absorption Rates	23
11 Position of Pituitary	28
12 Section of Oxy Pituitary	29
13 Action of Serum Gonadotrophin	32
14 Position of Adrenal	34
15 Section of Adrenal	35
16 Action of Adrenalin	36
17 Male Sex Organs	39
18 Testis	40
19 Effect of Testosterone on Capon's Comb	40
20 Female Sex Organs	41
21 Section of Ovary	42
22 Diagrammatic Representation of Normal Menstrual Cycle	45
23 Pregnenediol Excretion	46
24 Insulin Crystals	54
25 Cretinism	139
26 Typical Myxoedema	140
27 Typical Thyrotoxicosis	142
28 Cushing's Syndrome	151
29 Frohlich's Syndrome	152
30 Gigantism	153
31 Acromegaly	154
32 Dwarfism	155
33 Gynecomastia	163
34 Eunuchoidism	171

TABLES

I	Endocrine Glands and their Hormones	2
II	Effect of Alloxan on Blood Sugar	27
III	Hormones of the Anterior Lobe	29
IV	Action of the Pituitary Gonadotrophins on the Gonads	31
V	Amino acids in the Insulin Molecule	55
VI	Sterols	62
VII	Bile Acids	64
VIII	Sex Hormones and Excretion Products	70
IX	Œstrogen Content of Urine	72
X	Comparative Activity of Some Natural and Artificial Œstrogens	104
XI	International Standard Hormone Preparations	112
XII	Numbers of Animals required for Minimal Limits of Error in Assays	135
XIII	Relative Doses of Œstrogens	166
XIV	Identification Colours for Insulin Packs	178

INTRODUCTION

FOR many centuries glands have been known to anatomists but it is only within comparatively recent times that they have been recognised as organs which have the property of secreting chemical substances of great biological importance. Those which secrete directly into the blood stream are known as ductless glands, endocrine glands or organs of internal secretion and the substances which they secrete are called hormones. The importance of these organs began to be appreciated about the middle of the last century when it was found that destruction of the adrenal glands had far reaching effects in remote parts of the body. Endocrinology is the branch of science which deals with the functions and nature of these organs and their secretions. The chemical constitution of some hormones is known and many have been synthesised in the laboratory, other hormones resemble proteins and their exact chemical constitution is not yet known.

It is characteristic of the hormones that only minute quantities are required to produce profound physiological reactions in the body. The hormone of the adrenal medulla known as adrenaline is highly active when present in the blood in a concentration of less than one part in one hundred million. All the endocrine glands are characterised by having a plentiful supply of blood which can carry the hormones rapidly to all parts of the body. The main endocrine organs and their position in the human body are shown diagrammatically in the frontispiece. The hormones associated with each gland are listed in Table I.

The Ductless Glands The thyroid is found in all vertebrate animals. In man it takes the form of a pair of lobes situated on each side of the trachea at the base of the neck with a connecting band of tissue known as the isthmus. In the adult the gland weighs about twenty five grammes and its structure consists of a large number of vesicles lined with secreting cells and filled with a jelly like colloid. In conditions of over activity the microscopic structure undergoes a fundamental change. The hormone of the thyroid is the amino acid thyroxine, 3,5-diiodotyrosine is also found in the gland but this is regarded as a precursor in the biogenesis of the hormone. Both compounds have been synthesised. The secretions of the thyroid gland have a marked effect on the basal metabolic rate. Excessive activity of the gland speeds up all the vital processes, causes acute nervous tension and may result in exophthalmic goitre. A deficiency of the hormone in the adult results in the condition known as myxoedema which is characterised by a sluggish temperament and appearance and in children a congenital lack of the hormone results in cretinism.

The parathyroids are small, yellowish red organs which generally occur in pairs. They vary in size, number and position but in man

they are usually found on the inner side and towards the back of each lobe of the thyroid gland. The hormone appears to be a protein but little is known about its constitution. Its main function is to control the metabolism of calcium and inorganic phosphates.

TABLE I
ENDOCRINE GLANDS AND THEIR HORMONES

<i>Gland</i>	<i>Hormones</i>
Thyroid	Thyroxine
Parathyroid	A protein (not yet identified)
Pancreas	Insulin
Islets of Langerhans	
Pituitary	Growth Hormone
Anterior Lobe	Gonadotrophic Hormones
	(1) Follicle stimulating hormone
	(2) Luteinizing hormone
	(3) Lutetrophic hormone (Lactogen = hormone)
	Thyrotrophic Hormone
	Adrenocorticotrophic Hormone
Posterior Lobe	Oxytocic Factor
	Pressor Factor
Adrenal	
Medulla	Adrenaline
	Noradrenaline
Cortex	Numerous steroid ketones
	e.g. corticosterone, cortisone, deoxycortone
Gonads	
Ovaries	o (Estradiol)
Corpus Luteum	Progesterone
Testes	Testosterone

The parts of the pancreas known as the islets of Langerhans are responsible for the secretion of the protein insulin which regulates the assimilation of sugars and controls the concentration of glucose in the blood. The remainder of the pancreas produces enzymes which when introduced into the alimentary tract break down proteins, carbohydrates and fats. The islets of Langerhans are interspersed in the glandular tissue which produces these enzymes. Much work has been carried out on the chemistry of insulin and the size of the molecule and the nature and number of the amino-acids of which it is composed are now known with a fair degree of certainty although the order in which the several different amino-acids are joined together in insulin is not completely known.

The pituitary gland is composed of two distinct parts known as the anterior and posterior lobes. Each lobe arises from different embryonic tissues. In man it is situated in the centre of the head at the base of the brain. The whole gland is about the size of an acorn and usually weighs

rather less than one gramme. The anterior lobe is the source of a number of hormones which have far reaching effects on the functions of other glands especially the gonads. It has been asserted that the anterior lobe controls the activity of the whole endocrine system and may thus be regarded as the 'master gland'. Among the active principles arising from the anterior lobe are a growth promoting hormone, a thyroid stimulating hormone, gonadotrophic hormones, and a lactogenic hormone. The function of the posterior lobe is less clear, but it appears to secrete two hormones which contract the uterus and intestines respectively. Little is known of the chemical nature of these substances.

The adrenal glands or suprarenals are two in number. In man they weigh about four grammes each and are placed above and in front of the kidneys. Each gland consists of two parts, the medulla and the cortex. The medulla, which is the central portion, produces the hormones adrenaline and noradrenaline, which are basic derivatives of catechol. Adrenaline was the first hormone to be isolated in pure form and both adrenaline and noradrenaline have been synthesised by several methods. Adrenaline, which serves to mobilise the resources of the body to meet an emergency, causes acceleration of the heart beat, retardation of the digestive processes and liberation of the carbohydrate reserve. The cortex produces a large number of steroid hormones including corticosterone, dehydrocorticosterone, deoxycortone, cortisone, adrenosterone, and many others. Many of these have been synthesised from sterols or bile acids. In addition, small quantities of some of the sex hormones are found in the cortex. Certain of the hormones of the adrenal cortex are essential to life.

The gonads are the glands chiefly concerned with the secretion of the sex hormones. In the female they consist of the ovaries; in the male the testes. The cells of the ovaries and testes are responsible for secreting their hormones into the blood at a very early stage in human development, and this results in the development of female or male characteristics. During childhood further sexual differentiation is to a large extent arrested, to be resumed with greater effect at puberty until full maturity has been reached, usually in the early twenties. The ovaries of the adult woman are a pair of egg-shaped organs situated below the fallopian tubes in the upper segment of the pelvis. At approximately monthly intervals from puberty to the menopause, an ovum is discharged from its follicle in the ovary. This ruptured follicle then becomes filled with luteal cells containing a yellow fatty substance and finally a new small discrete organ, known as the corpus luteum, projects from the surface of the ovary. The ovaries produce the female or follicular hormone known as α -oestradiol, which is classified as an oestrogen, while the corpus luteum produces a second hormone, progesterone. Both hormones are steroid in character and have been synthesised from sterols in the laboratory. Other closely related substances such as oestrone, oestriol, and pregnanediol are found in human

female urine especially during pregnancy. These are the forms in which the hormones are excreted. The testes of the adult man are paired ovoid organs of about the size of a small hen's egg and are contained in an extension of the body cavity known as the scrotum. The hormone of the testes is testosterone — a steroid of known constitution which can be prepared from cholesterol. This hormone is termed an androgen and other androgens such as androsterone and dehydroisoandrosterone which are the metabolic products of testosterone to which they are closely related chemically have been isolated from male urine.

The endocrine glands do not in general function in isolation and independently of each other and different glands may affect biological processes in different ways. Whereas the thyroid hormone stimulates the oxidation of sugars, adrenaline liberates sugar from the liver and insulin controls the assimilation of sugars from the blood. The different hormones may therefore either antagonise or reinforce the action of each other. Of even greater importance are the more direct influences which the secretions of one gland have on the functions of others and this is best illustrated by the powerful and fundamental effects throughout the whole endocrine system which can be traced to the anterior lobe of the pituitary.

CHAPTER I

HISTORY

THE origin of endocrinology can be traced back to the earliest times when man attributed special virtues to the internal organs obtained from slain enemies or animals but modern interest in hormones is often said to date from the sensational experiments of Brown Sequard reported before the Societe de Biologie in Paris in 1889. At the age of seventy-two he treated himself for declining vigour with injections of aqueous extracts of the testes of dogs and guinea pigs but the beneficial results which he claimed can be attributed only to over enthusiasm and auto-suggestion. Many years earlier in 1849 Berthold had carried out experiments on the castration of male chicks which resulted in the development of capons large sexless birds without comb wattles spurs and other accoutrements of avian masculinity further when testes were transplanted under the skin of the capons the masculine characteristics developed. Although the analogy between the testes and the ovaries was recognised by early anatomists the changes which result from the removal of the ovaries were discovered much later than those resulting from the removal of the testes and it was not until towards the end of the nineteenth century that it was also found that the transplantation of ovaries into castrated animals resulted in the temporary reversal of the changes brought about by castration. The failure of attempts to treat male deficiencies and more particularly to effect rejuvenation by means of the oral administration of dried gland tissue or by means of the parenteral administration of aqueous extracts led naturally to the experiments on the grafting of animal testicles by Voronoff and indirectly to the device of the ligation of the vas deferens by Steinach but both ventures in spite of early claims were doomed to failure as a solution to the many sided problem of senescence. Both the grafting of testicular tissue and the tying of the vas deferens were designed to increase the concentration of the male hormone.

Adrenals The first insight into the functions of the adrenal glands was provided by Addison who in 1849 showed that their destruction led to the disease which now bears his name. This disease which is characterised by vomiting bronzing of the skin and muscular weakness can now be treated successfully by administration of one of the hormones found in the adrenal cortex. Active chemical work on the nature of the compounds present in the cortex followed much later and dates from about 1930. Already some twenty eight different steroids have been isolated and identified from this source mainly in the laboratories of Reichstein in Switzerland and of Kendall and Wintersteiner in America. Among these is cortisone the successful use of which in the treatment of rheumatic diseases was first reported by

Hench and Kendall in 1949 The first observations on the functions of the adrenal medulla are usually attributed to Oliver and Schafer who in 1894 observed the effects on man of extracts of the adrenal glands which among other reactions cause an increase in blood pressure. In 1901 the active principle *adrenaline* was isolated in pure form and three years later it was the first hormone to yield to synthesis in the laboratory. The occurrence of *noradrenaline* in the medulla was reported in 1949.

Thyroid In 1856 Schiff found that dogs and guinea pigs could not survive the removal of their thyroid glands and that the animals could be saved by transplanting the glands at any point under the skin. The fact that excessive thyroid secretion might be the cause of *exophthalmic goitre* was first suggested by Rehm in 1884. These observations not only established the importance of the gland but also led to the successful use of gland extracts in the treatment of thyroid deficiency as revealed in *cretinism* and *myxoedema*. The isolation of the active principle in pure form was not achieved until 1919 eight years later the synthesis of *thyroxine* was reported by Harington and Barger.

Pancreas The recognition of some connection between sweet urine and the pancreas dates back to the early years of surgery but the cause of *diabetes mellitus* remained enveloped in mystery until 1889 when von Mehring and Minkowski observed that the removal of the pancreas from dogs resulted in the production of urine very rich in sugars. The dogs became weak and finally died after having shown all the symptoms associated with *diabetes mellitus*. In this way the function of the pancreas became associated with *diabetes mellitus* and this suggestion was confirmed when it was observed that the pancreas of human patients who had died as a result of *diabetes mellitus* showed signs of damaged cells. Many attempts were made to isolate from the pancreas an active principle which could be administered to diabetic patients but success did not come until 1919 when Banting showed that the active principle to which the name *insulin* was given was sensitive to and destroyed by the *trypsin* in the pancreatic juice. By tying the pancreatic ducts in dogs and allowing a period of time for the degeneration of the existing *trypsin* Banting and Best were able to prepare for the first time extracts which could relieve *diabetes mellitus* when administered by subcutaneous injection and it was in 1922 that the first human patient was treated successfully with insulin.

Parathyroids In the decade which followed the discovery of insulin attention was directed to the isolation of the active principles of many other glands and Collip and Hanson isolated from the parathyroid glands a potent extract which was shown to have the property of preventing tetanic convulsions and of controlling calcium metabolism.

Pituitary At about the same time important developments in connection with the pituitary gland were reported by Evans and Long. From the anterior lobe they isolated an extract which when injected subcutaneously into rats caused rapid and abnormal growth and brought about fundamental changes in sex development. It was later demonstrated by Smith that normal growth was resumed when crude anterior lobe extracts were administered to dwarfed hypophysectomised rats. The relation between the pituitary and sex maturity was confirmed when Aschheim and Zondek showed that a similar active principle the gonadotrophic factor was present in human pregnancy urine an observation which later led to the development of the Aschheim Zondek test for pregnancy. A new source of gonadotrophic hormones was discovered by Cole and Hart in 1930 in the blood serum of pregnant mares. The exact number of active principles associated with the gonadotrophic factor is uncertain but at least two different hormones are usually recognised namely the follicle stimulating hormone and the luteinising hormone. A relation between the pituitary and thyroid glands was first suggested by Rogowitsch in 1888 and confirmed by later workers. Evidence was also obtained of the existence of an adrenocorticotrophic substance and of a lactogenic or lactation hormone (prolactin). The anterior lobe of the pituitary thus affords a considerable variety of factors all of which appear to be proteins. The complete separation of the different factors from each other is a problem beset with great practical difficulties which are further complicated by a lack of precise knowledge of the extent to which activity is due to a specific hormone or to a combination of hormones. The relation between the posterior lobe of the pituitary and diabetes insipidus was recognised by Vassile and Sacchi in 1893 and in 1906 Dale observed that an extract of the posterior lobe could cause contraction of the muscle of the uterus.

Gonads The remarkable advances which have been made in our knowledge of the sex hormones which with the hormones of the adrenal cortex may be classified as the steroid hormones began in 1923 when Allen and Doisy demonstrated that the follicular fluid obtained from animals ovaries contained an active oestrogenic hormone which six years later was isolated in pure crystalline form from human pregnancy urine. Shortly afterwards a second hormone oestriol was isolated by Marnian to be followed later by the isolation in Doisy's laboratory of α oestradiol from the follicular fluid of sow ovaries. α Oestradiol had previously been prepared in the laboratory by Schwenk and Hildebrandt by the reduction of oestrone. As early as 1903 Fraenkel had observed that the removal of the corpora lutea from rabbits in early pregnancy was followed by abortion but it was not until 1928 that evidence was obtained by Weichert of the existence of a specific corpus luteum hormone. This hormone progesterone was isolated in pure

form by Butenandt in 1934 and was synthesised in the same year. In the male hormone field Butenandt reported in 1931 the isolation of androsterone from male urine and later work by Koch and McGee in Chicago and by Laqueur in Amsterdam led to the isolation of the male hormone testosterone. Soon after its discovery androsterone was prepared in the laboratory from cholesterol by both Ruzicka and Butenandt and this achievement was shortly afterwards followed by the preparation of testosterone itself.

Chemical Constitution It will be seen that as with the vitamins the hormones do not belong to any one specific class of chemical compound. Some like adrenaline and thyroxine are relatively simple derivatives of benzene, others including all the sex hormones as well as the hormones of the adrenal cortex belong to the steroid group, yet others such as insulin and the hormones of the pituitary are typical proteins. Adrenaline and thyroxine being the hormones of simplest constitution were the first to be synthesised, but the more complex sex hormones and hormones of the adrenal cortex have also been synthesised by many different methods which make use of either sterols, bile acids or more recently sapogenins as starting materials. These are only partial syntheses in that they start with a preformed steroid nucleus, but in one or two cases the total synthesis of a steroid hormone has been achieved in spite of the complexity of the stereochemical difficulties which have to be overcome. The total synthesis of all the steroid hormones may now be said to be within sight of achievement. On the other hand exact knowledge of the constitution of the protein hormones is still lacking and their synthesis remains a distant objective. In some instances, notably with the oestrogenic hormones, considerable success has been obtained in the preparation of artificial compounds which appear to possess all the properties of the natural hormones and many such preparations have been introduced into medical practice.

Clinical Applications It is obvious that there is now an extensive fund of knowledge, anatomical, physiological and chemical, on the nature and functions of the endocrine glands and their hormones and the study of endocrinology is recognised as an essential part of the medical curriculum. Its practical application in the treatment of disease has many notable achievements to its credit, but not infrequently difficulties have arisen in applying to human patients the results obtained with experimental animals. Such difficulties, however, are not confined to this field alone and are frequently encountered in chemotherapy as well as in other branches of medical research. Perhaps the most successful applications in clinical practice are to be found in the uses of insulin, thyroxine and adrenaline. The use of certain sex hormone preparations and the treatment of Addison's disease with deoxycortone acetate have also been conspicuously successful.

CHAPTER II

PHYSIOLOGY

WHEN considering the physiology of the hormones of the endocrine glands it is important to take into account the interrelation that exists between the different hormones and because of the dependence of one gland upon another a logical approach to the subject is difficult. For proper physical and mental development all the endocrine glands in the body must be functioning and a delicate balance of the amount of hormone secreted by each gland must be maintained.

Zondek¹ states that hormones mutually promote or inhibit one another inasmuch as each individual hormone may stimulate or inhibit the activity of other hormonal glands. In other words an under or overactive gland leads to changes in other glands and the effects of dysfunction must be considered in relation to the endocrine system as a whole.

THYROID

The thyroid consists of two lateral lobes together weighing about twenty five grammes in a human adult which are joined together by the isthmus a narrow piece of similar tissue. It is situated in the neck on the upper portion of the trachea and produces the hormone thyroxine.



FIG. 2

POSITION OF THYROID

The two lobes of the human thyroid lie in the neck on either side of the larynx and upper trachea. The isthmus which connects them lies in front of the upper four rings of the trachea.

Hypothyroidism The thyroid regulates the production of energy and controls physical and mental development. The result of an underactive thyroid gland is myxoedema or hypothyroidism. Congenital absence of the gland causes cretinism. In adults thyroid insufficiency is indicated by well marked and typical symptoms: all the metabolic processes of the body are slowed down, the skin becomes dry, coarse and puffy, the hair becomes brittle and coarse, and there is general lethargy. In children there is a retarded state of development, both physical and mental, the heart rate is slow, and the children are generally apathetic. The administration of thyroid by mouth results in a dramatic improvement in myxoedema, but in cretins thyroid therapy to be effective must be instituted early in life.

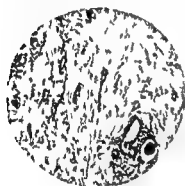


FIG 3

SECTIONS OF THYROID

A Myxoedematous tissue showing reduced vesicles scattered in fibrous tissue ($\times 400$) *B* Normal tissue showing vesicles filled with gelatinous colloid embedded in connective tissue ($\times 400$) *C* Toxic goitre tissue showing increased vascularity with many vesicles containing little colloid ($\times 400$)

Hyperthyroidism An overactive thyroid leads to hyperthyroidism or primary thyrotoxicosis (toxic goitre). Other names given to diseases associated with an overactive thyroid gland are Graves, Parry's or Basedow's disease and exophthalmic goitre. Authorities differ as to the best nomenclature. Exophthalmos (bulging of the eyes) is not always present and the term most frequently used is Graves' disease.

Sir Robert Graves first described the symptoms in 1825 before the effects of the administration of thyroid had been observed. The symptoms of this condition are an increase in the heart rate and a general state of anxiety and restlessness sometimes especially in severe cases accompanied by exophthalmos.

Microscopical Characters The thyroid gland as shown by a microscopical examination of a section consists of a large number of vesicles embedded in connective tissue and filled with a viscous gelatinous material generally referred to as colloid which passes into the blood stream either directly or through the lymphatic system. The amount of colloid excreted is regulated by the thyrotrophic hormone of the anterior lobe of the pituitary. Microscopical examination of the gland in myxoedema shows delicate fibrous tissue in which the remains of vesicles are scattered. In hyperthyroidism the thyroid shows increased vascularity with many vesicles containing little or no colloid.

Thyroid and Metamorphosis Minute amounts of thyroid greatly accelerate the change of tadpoles into frogs. The accelerated metamorphosis occurs when the tadpoles are kept in water containing thyroid or thyroxine and is most useful as a test in the biological investigation of compounds for thyroid activity. Substances other than

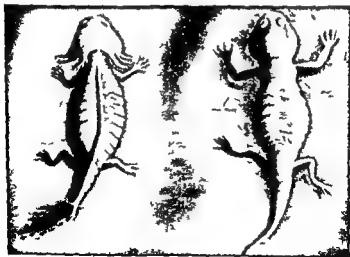


FIG. 4

INDUCED METAMORPHOSIS IN AXOLOTL

The Mexican salamander (*Ambystoma mexicanum*) or axolotl usually undergoes no metamorphosis but breeds in the gilled state (left) when fed on thyroid it develops a terrestrial form (right).



FIG 5

SOUTH AFRICAN CLAWED TOAD (*Xenopus laevis*)

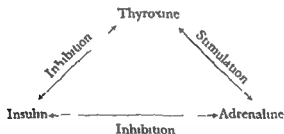
The development from tadpole to toad is normally complete in 10 to 12 weeks but may be hastened by substances containing thyroxine such as thyroid and iodinated casein. The photographs show tadpole (top left) intermediate stage (top right) male toad (bottom left) and female toad (bottom right). The female toad is also used in the *xenopus* (Hogben's) pregnancy test.

thyroxine however are able to produce in mammals a response similar to that given by thyroid feeding and the effect on tadpoles does not provide a satisfactory method for the assay of thyroid preparations

Frogs formed by feeding with thyroid are however dwarfs and soon die since thyroid accelerates their metamorphosis but inhibits their growth. It has been shown that the thyroids from various forms of goitre differ greatly in their specific influence on the tadpole.²

Another example of the action of the thyroid hormone is seen when the Mexican salamander or axolotl is fed on thyroid. In the normal state the axolotl does not usually undergo metamorphosis but the administration of thyroid or thyroxine induces a metamorphosis resulting in a land animal. Iodine alone is incapable of inducing such a change since the axolotl has an undeveloped thyroid.

Thyroid and Other Endocrine Glands The following diagram indicates a possible relation between the thyroid and other endocrine glands —



The thyroid hormone for example is thought to be necessary for the normal action of adrenaline and it is likely that an excess of thyroxine either stimulates the production of extra adrenaline or sensitises the tissues to its action. Thyroxine and insulin are in certain respects antagonistic and a person with an underactive thyroid has an abnormally high sugar tolerance. Diabetes mellitus is frequently associated with increased activity of the thyroid. Thyroid enlargement may occur at puberty, at the climacteric and also during pregnancy and menstruation. This may be due to an increased secretion of the ovarian hormones or to a deficiency of iodine brought about by increased metabolism at these times. Thyroidectomy frequently causes degeneration of the gonads in males and females.

Basal Metabolic Rate In the diagnosis of thyroid disorders a determination of the heat production capacity or basal metabolic rate (B.M.R.) is most valuable. The standard is the heat production of a person in a fasting condition at rest and the basal metabolic rate is expressed as a percentage above or below the normal after taking age

height and weight into consideration. When the heat produced is greater than normal the basal metabolic rate is expressed as plus and when less than normal minus. In mild hyperthyroidism patients have a basal metabolic rate of +10 to +30 and in severe hyperthyroidism +30 to +60. Other conditions such as fever can elevate the basal metabolic rate appreciably but they can usually be easily differentiated from a raised basal metabolic rate due to hyperthyroidism.

In experimental animals the basal metabolic rate may be found by measuring the heat lost in a calorimeter but in man it is determined indirectly by finding the oxygen consumption over a period in a Benedict Roth apparatus. This consists of a tank filled with oxygen suspended in water and connected by means of tubing to the patient's mouth. The patient inhales oxygen directly from the tank and exhales back to the tank through a soda lime tower where the carbon dioxide formed is removed. The decrease in volume of oxygen in the tank is thus a direct measure of oxygen consumed. Before the estimation the patient should fast for twelve hours rest for half an hour and be unexcited³.

The basal metabolic rate yields valuable information in conditions caused by disturbances of the thyroid and pituitary glands. In exophthalmic goitre it is increased by an amount varying proportionately with the severity of the condition. It has also been shown that the basal metabolic rate is lowered for fourteen days in patients with hyperthyroidism by the administration of Lugol's solution (Aqueous Solution of Iodine B.P.) and that it then rises again. In hypothyroidism the basal metabolic rate is -20 to -40.

Radioactive Iodine. As a means of studying the absorption and elimination of drugs in the body radioactive elements are proving to be most useful particularly when the estimation of minute amounts by chemical means is impossible. Comparatively little is known of the fate of thyroxine and iodine in the body but it has been shown by administering radioactive iodine that more than 50 per cent. of the amount administered appears in the thyroid gland. In hypothyroidism the thyroid gland utilises little or no iodine; in simple goitre more iodine is taken up and in toxic goitre or hyperthyroidism although more iodine is absorbed it is not retained. This fact may give a clue to the reason why iodine causes a temporary remission of symptoms in toxic goitre. Radioactive iodine has also been used therapeutically for the selective destruction of the active tissue of the thyroid gland by internal radiation when it acts in a manner similar to that of X rays⁴. It is claimed that a cure rate of 80 per cent. can be obtained in patients with hyperthyroidism by treatment with radioactive iodine and it may be that when more precise details have been worked out this method will replace the use of drugs and the surgical removal of a portion of the thyroid gland.

Iodinated Proteins Many attempts have been made to prepare physiologically active iodinated proteins which can be used instead of thyroid. Rivers and Randall¹ prepared iodinated ox plasma tridein (a mixture of proteins from ground nuts) and casein. Iodinated casein proved to be the most satisfactory and it was possible by administering it to cows to raise their basal metabolic rate and stimulate the production of an increased yield of milk. This use is still under experimental study and cannot be generally recommended until more detailed information is available regarding possible undesirable effects which may follow administration over a long period. The activity of these compounds has been shown to be due to the presence of thyroxine and this adds further evidence in proving that thyroxine is the hormone of the thyroid gland.²

Antithyroid Substances In 1928 it was shown by Chesney and his co-workers³ that ingestion of plants belonging to the genus *Brassica* (family Cruciferae) especially cabbages produced thyroid enlargement with associated underactivity in rabbits and in 1941 MacKenzie, MacKenzie and McCollum⁴ while working on the effects of sulphaguanidine in rats discovered that it gave rise to hypothyroidism. Later MacKenzie and MacKenzie⁵ and Astwood *et al*¹¹ showed that other sulphonamides also caused hyperplasia of the thyroid gland in rats and many compounds were examined with a view to finding one having an antithyroid action.

Astwood¹² showed that thiourea and thiouracil were capable of reducing the secretion of the thyroid gland and subsequent studies have led to the belief that they act by preventing iodine from combining with tyrosine and consequently the synthesis of thyroxine. Subsequently methylthiouracil was shown to exert a more rapid therapeutic action in smaller doses than thiouracil^{13, 14} while Astwood *et al*¹⁵, Anderson *et al*¹⁶ and Pavin and Coodechild¹⁷ have found that of over three hundred drugs tested in the rat propylthiouracil has the greatest antithyroid activity. As a result of clinical work with propylthiouracil Astwood and Vander Laan¹⁸ have reported a satisfactory control with no untoward reactions in fifty-two patients. The administration of thiouracil and its derivatives requires to be carefully controlled since they are liable to give rise to toxic symptoms, agranulocytosis the most dangerous of these mostly occurs from overdosage. They should not be used in the treatment of simple goitre when the enlargement of the thyroid is due to underactivity of the gland nor should they be used in the treatment of large toxic goitres which are causing pressure symptoms.

PARATHYROID

The parathyroid glands are the smallest members of the endocrine system. In man they consist of yellowish red ovoid bodies 3 to 15 millimetres in length, 2 to 4 millimetres in width and 1 to 2 millimetres

in thickness. The average combined weight of the glands is about 0.5 gramme. The number and position of the parathyroid glands is extremely variable; usually four are situated on the under surface of the thyroid, but as many as twelve have been reported.

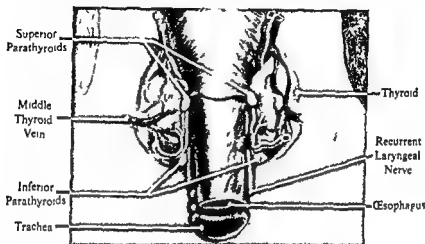


FIG. 6

POSITION OF PARATHYROIDS

The four small glands lie on either side of the thyroid with which they have no vascular connection.

The name *parathyroid* is somewhat misleading since these glands have no vascular connection with the thyroid. Although they may influence and be influenced by other glands, the existence of a parathyrotrophic hormone in the anterior lobe of the pituitary has not been proved, and other glandular interrelations are not clear.

Effects of Parathyroidectomy The accidental removal of the parathyroids in an operation on the thyroid proved that they were essential to life, and experimental work on animals shows that they are associated with calcium and phosphorus metabolism. The complete removal of the parathyroid glands is followed by muscular tremors, cramps, and convulsions, ultimately leading to the death of the animal. The symptoms are associated with a fall in the calcium content of the blood serum and a retention of acid-soluble phosphates. Parathyroidectomised animals can, however, be kept alive by feeding on a high calcium and low phosphorus diet.

Hypoparathyroidism In man, under-secretion of the parathyroid hormone, or hypoparathyroidism, is associated with tetany, the chief symptoms of which are increased nervous excitability and

painful muscular spasms. Ectodermal lesions such as loss of hair, brittle nails and cataract may occur in patients suffering from long standing tetany. Tetany due to hypoparathyroidism is a somewhat rare occurrence. It most frequently arises from injury or removal of parathyroid tissue. Surgical removal may occur accidentally during thyroidectomy on account of the proximity of the parathyroid and thyroid glands. Tetany is also associated with rickets and sometimes with pregnancy and lactation owing to the increased calcium requirements at these times.

Hyperparathyroidism : Over secretion of parathyroid hormone which may follow a tumour of the gland is characterised by osseous decalcification resulting in softening, rarefaction and deformity of the bones. There is pain in the bones and other tissues and renal calculi are commonly present. In the absence of surgical treatment the progressive deformity and weakness may lead to death.

Calcium and Phosphorus Metabolism : Since the parathyroid hormone is so intimately concerned with calcium and phosphorus metabolism it is important to consider the function of these elements in the body.

The absorption of calcium and phosphorus from the bowel depends primarily on the calciferol (vitamin D) content of the food, the calcium phosphorus ratio and the hydrogen ion concentration of the bowel contents. Increased acidity in the bowel favours absorption and conversely increased alkalinity gives rise to insoluble calcium salts which are not absorbed. If the phosphorus intake is excessive, insoluble calcium phosphate is formed and calcium loss occurs. The blood serum normally contains about 10 milligrams of calcium and 3 to 5 milligrams of inorganic phosphate per 100 millilitres. Should the calcium content fall to 8 to 10 milligrams per 100 millilitres with a proportionate increase in phosphorus then tetany results. The skeleton which contains over 99 per cent of the body's calcium acts as a storehouse for calcium and phosphorus from which these elements can be mobilised as required for the blood serum. Its mode of action is in some ways comparable to that of the liver which stores carbohydrate as glycogen and releases it as glucose when required.

The rate of deposition of calcium in the bones depends upon the calcium ingestion and on the amount of parathyroid hormone present in the blood. The parathyroid hormone mobilises calcium from the bones and maintains the correct level of phosphorus in the body fluids and cells.

Albright and Reifenstein¹⁹ believe that the primary action of the hormone is on phosphorus metabolism and the changes which occur in the calcium content of the blood serum are secondary to those of phosphorus metabolism.

Neufeld and Collip⁹ consider that the parathyroid hormone has no direct effect on the bone its primary action being on the excretion of phosphates by the kidneys Albright¹⁰ discussing drugs which influence calcium and phosphorus metabolism points out that vitamin D favours their intestinal absorption while the parathyroid hormone regulates their concentration in the blood serum it does not affect the absorption of calcium and phosphorus in the bowel A phosphatase enzyme is also concerned in bone formation It is believed to be a product of proliferating cartilage cells Alkaline phosphatase has a maximum activity between pH 9 and 10 while an acid phosphatase which has been isolated from prostatic epithelium has a maximum activity at about pH 4.8 Serum alkaline phosphatase is at a minimum in normal healthy adults and increases whenever bone is being formed or rapidly destroyed in such conditions as hyperparathyroidism Magnesium greatly increases the activity of the enzyme whereas calcium ions are mildly inhibitory

Parathyroid Hormone and Tetany The parathyroid hormone raises the calcium content of the blood serum and will thus relieve tetany following parathyroidectomy Its use in man for this purpose however has the disadvantage that it takes about four hours to act and injections may cease to be effective after two or more weeks owing to the supposed development of antihormones

Robertson² discussing the various methods used in the treatment of parathyroid tetany refers to the dangers of overdosage loss of effectiveness or decalcification of the bones if parathyroid preparations are used over long periods He found that thyroid or thyroxine temporarily controlled tetany and concludes that calcium salts either by injection or orally still remain the chief therapeutic measure Shelling³ found that following an injection of 10 millilitres of a 10 per cent solution of calcium gluconate there was a rapid increase in the percentage of serum calcium (up to 100 per cent increase at the end of injection) The serum calcium fell however to the previous level in two to four hours

Dihydratachysterol and Calciferol In recent years interest has been aroused in a substance known as dihydratachysterol or AT 10 (anti tetany 10) a by product formed during irradiation of ergosterol in the manufacture of calciferol This compound was first isolated and used clinically by Holtz in 1927 who reviewed his results in 1941¹¹ It is administered by mouth in solution in oil and will abolish tetany in two to three days slowly raising the serum calcium to normal levels Dihydratachysterol is considered to have an effect intermediate between that of calciferol and the parathyroid hormone In its method of action however it appears to resemble the parathyroid hormone more than calciferol and it has no appreciable antirachitic effect

Sevringhaus and St John ⁵ found the oral administration of large doses of calciferol combined with calcium salts effective in treating tetany and consider that calciferol is apparently as safe as dihydrotachysterol. A dose of 100 000 units of calciferol daily for two weeks raises the serum calcium level but is without effect on the inorganic phosphorus.

Brack ⁶ has made the interesting observation that in a patient who had developed tetany following thyroidectomy and had received treatment with dihydrotachysterol and calcium intravenously the tetany could be controlled with a very much smaller dose of dihydrotachysterol without calcium if a histamine antagonist were administered at the same time. No explanation of the mode of action is suggested.

PANCREAS

The pancreas or sweetbread is a relatively large gland analogous in structure to the salivary glands but softer and less compactly arranged. It is situated in the upper abdomen and weighs about 75 grammes.

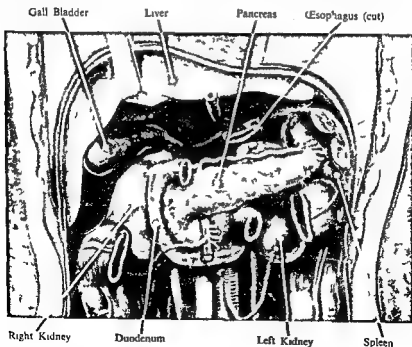


FIG 7

POSITION OF PANCREAS

This large gland (approximately 7 in. by 1½ in.) lies in the upper abdomen behind the stomach and extends some what horizontally from the duodenum to the spleen.

The pancreas is a compound organ consisting of cells which produce enzymes which aid digestion interspersed throughout the gland are clusters of cells called islets of Langerhans which are responsible for the secretion of the hormone insulin. These small islets form a thirtieth to a hundredth of the mass of the pancreas and it is generally considered that the acini which secrete enzymes and the islet tissue are distinct one producing an external secretion which is conveyed through the pancreatic ducts into the duodenum while the other produces insulin which is carried directly into the blood stream by small capillaries in the centre of the islet.

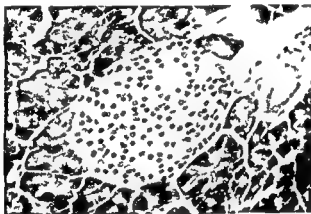


FIG. 8

SECTION OF MAMMALIAN PANCREAS

Showing an islet of Langerhans. The islet cells are responsible for the secretion of insulin which passes directly into the blood stream.

Two types of cells are present in the islets alpha cells whose granules are insoluble in alcohol and beta cells whose granules are soluble in alcohol. It has been shown that the beta cells are those which secrete insulin.

Carbohydrate Metabolism The chief sources of bodily energy are sugars, starches and glycogen. Carbohydrates are usually ingested as starches and disaccharides and these are converted into monosaccharides in the body and then into glycogen which is stored in the liver and muscles. The liver can also convert amino acids into glycogen and it acts as a storehouse from which the body obtains glucose as required by the action of the enzyme glycogenase in the liver. About 500 grammes of glycogen are stored in the body, 100 grammes being stored in the liver and most of the remainder in the skeletal muscles. Insulin is essential for the correct metabolism of carbohydrates and

fats and the most characteristic effect of an injection of insulin is to reduce the amount of sugar in the blood

Action of Insulin Insulin has three main actions: it aids the combustion of glucose by the tissues; it favours the formation of glycogen in the liver and muscle; and it inhibits glucose formation from amino acids in the liver. When there is a deficiency of insulin, sugar appears in the urine and diabetes mellitus results. This is however only an indication of a complicated derangement in metabolism which is taking place: glycogen reserves in the liver are rapidly depleted and when they are exhausted body proteins are utilised. In the later stages fat is drawn upon, resulting in fat derivatives or ketone bodies such as acetone making their appearance in the blood and urine.

If diabetes mellitus is untreated, diabetic coma may result. It may also occur in diabetic patients following infections such as influenza or pneumonia owing to an increased insulin requirement in these conditions. Coma is due to gross impairment of the fat metabolism resulting in the formation of ketones which exert toxic action particularly upon the central nervous system. Ketone bodies including acetoacetic acid, β -hydroxybutyric acid and acetone appear in the urine and the characteristic sweet smell of acetone can usually be detected in the patient's breath.

Blood Sugar Levels There is no reliable method for the determination of the amount of insulin in small quantities of blood and the diagnosis of diabetes mellitus is usually made by estimating the amount of glucose in the urine and in the blood. The normal fasting blood sugar level is about 100 milligrams per 100 millilitres; it rises to 130 to 170 milligrams per 100 millilitres after a meal, returning to normal in about two to three hours. Thus although 200 grammes of glucose may be absorbed after a meal, the rise in blood sugar represents an increase in circulating glucose of only about three grammes. This constancy is lacking in severe untreated diabetes where the fasting level may be 130 milligrams per 100 millilitres, rising to 200 to 500 milligrams per 100 millilitres and remaining above 180 milligrams for the greater part of the day. Below a certain blood sugar level, known as the leak point or renal threshold, no sugar is excreted by the kidneys. When the blood glucose level exceeds this threshold, glucose passes through the kidneys and into the urine. In some people the renal threshold is below 180 milligrams per 100 millilitres and sugar appears in the urine even though the blood concentration is not abnormally high. This condition is known as renal glycosuria or diabetes innocens and requires no treatment. Lawrence and McCance²⁷ point out that renal glycosuria may be the cause of a diagnostic error if the possibility of a low renal threshold is not considered in patients presenting glycosuria without typical symptoms of diabetes mellitus.

Glucose Tolerance Test To confirm the diagnosis of diabetes mellitus blood sugar examinations are frequently carried out before and after the administration of glucose and this test is called a glucose tolerance test. The patient is allowed an unrestricted carbohydrate diet for several days and a blood sugar determination is made in the morning after the patient has fasted since the evening meal of the previous day. The patient then drinks a glass of water containing 50 grammes of glucose and further blood sugar determinations are made at half hourly intervals for two hours. Samples of urine are also collected and tested for sugar and ketone bodies. The blood sugar values are plotted and Fig. 9 illustrates the difference between the blood sugar concentration of a normal person and of a person with diabetes mellitus.

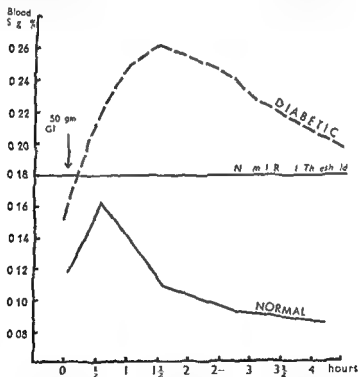


FIG 9

GLUCOSE TOLERANCE TEST

The graphs indicate the changes in the blood sugar levels following the administration of 50 grammes of glucose to a normal and a diabetic subject.

Insulin Preparations The type of preparation which has been longest in use consists of a simple solution from which however the insulin is so rapidly absorbed by the body that two or more injections are needed each day to maintain the action of the insulin. Attempts

have therefore been made to modify the action of insulin in order to give a slow prolonged effect. The first real advance was achieved in 1936 and 1937 by Hagedorn^{20, 21} who found that protamine obtained from the sperm of salmon trout when mixed with insulin and a suitable buffer to give a final reaction of pH 6.2 produced a precipitate of protamine insulin which had a low solubility in the tissue fluids and was only slowly absorbed. Scott and Fisher^{22, 23} found that the addition of a very small percentage of zinc to the insulin and protamine prolonged their action. The zinc also stabilised the protamine insulin suspension so that it was possible for it to be issued in one vial ready for injection. This preparation known as protamine zinc insulin has superseded protamine insulin.

In 1937 Bayn and Broom^{24, 25} investigated the effect of different substances on the absorption of insulin and showed that whereas small concentrations of metal produced a prolongation of hypoglycaemia increasing the amount of metal present eventually resulted in a complete inhibition of the normal insulin response. They also confirmed the observations of Bischoff²⁶ and Gray²⁷ that tannic acid prolongs the hypoglycaemic action of insulin and that the addition of zinc to the tannic acid insulin complex further prolongs insulin hypoglycaemia.

Reiner *et al*²⁸ have prepared a combination of insulin with globin, a protein obtained from the red blood cells of the ox by removal of the chromogen fraction with the addition of a trace of zinc chloride to the mixture. They found that globin zinc insulin had an action which was intermediate in intensity and duration of effect between soluble insulin and protamine zinc insulin.

The relative action of the three insulins is shown in Fig. 10.

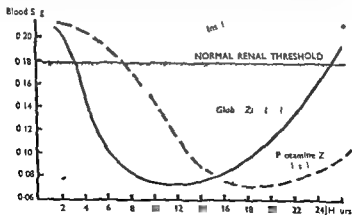


FIG. 10

INSULIN ABSORPTION RATES

The rapid absorption of insulin, the more prolonged absorption of globin zinc insulin and the low duration of effect of protamine zinc insulin are shown by the three curves on the graph.

Biochemical Examinations In diabetes mellitus biochemical examination of the blood for sugar and of the urine for sugar and acetone bodies or ketones is essential in diagnosis and for ascertaining the amount of insulin required for treatment. The determination of glucose in the blood and urine both depend upon the fact that glucose in alkaline solution is a powerful reducing agent.

Sugar in Urine—Qualitative Tests (1) *Benedict's Test* Urine examinations for sugar can be carried out easily by a diabetic patient using Benedict's qualitative reagent which is prepared as follows—

Sodium Carbonate	200.0 g
(or Exsiccated Sodium Carbonate)	90.0 g)
Sodium Citrate	173.0 g
Copper Sulphate	17.3 g
Distilled Water	to 1000.0 ml

Dissolve the sodium carbonate or the exsiccated sodium carbonate in 600 ml of water with the aid of gentle heat. Add the sodium citrate, cool and with constant stirring slowly add a cold solution of the copper sulphate in about 100 ml of distilled water. Add sufficient distilled water to produce the required volume.

To carry out the test 5 ml of Benedict's qualitative solution is added to 8 drops of urine in a test tube. The mixture is boiled for two minutes and allowed to cool. If glucose is present the reagent changes from clear blue to green, yellow or brick red. On standing reduced copper settles to the bottom of the tube as a reddish yellow deposit. 0.1 per cent of sugar gives a very slight reduction and the fluid turns deep blue on standing. 1 per cent of sugar produces a heavy precipitate but there is still some blue colour in the supernatant liquid.

(2) *Fermentation Test* To confirm that the reducing substance present in the urine is glucose a fermentation test may be carried out. A trace of tartaric acid is added to a sample of the urine. The specific gravity is determined and some baker's yeast is added. The mixture is allowed to stand in a warm room for twenty-four hours and the specific gravity again determined. If glucose is present fermentation will have taken place and there will be a fall in the specific gravity. An alternative method is to ferment the urine in an inverted test tube or a Doremus ureometer and collect the carbon dioxide produced by the fermentation of the glucose. It is advisable to put on control tubes: one with yeast and glucose to make sure that the yeast is active and one with yeast and normal urine to make sure that the yeast contains no glucose.

Sugar in Urine—Quantitative Tests More precise quantitative methods for determining the amount of glucose in the urine are only occasionally required and are described fully in standard textbooks. In Benedict's quantitative test the volume of urine which is required to dispel the blue colour from 25 ml of the reagent is determined, the urine being diluted if necessary so that the volume of diluted urine required is about 10 ml. The colour of 25 ml of Benedict's quantitative

reagent is dispelled by 0.05 g of glucose and from the figures obtained the percentage of glucose in the urine may be calculated

Ketone Bodies in Urine Two tests are available for detecting acetone bodies in urine. Rothera's nitroprusside test which is extremely sensitive and shows the presence of both acetone and acetoacetic acid and Gerhardt's ferric chloride test which if positive indicates dangerous acidosis requiring immediate treatment with insulin.

(1) Rothera's Nitroprusside Test An excess of ammonium sulphate is added to a test tube half full of urine followed by a few drops of a freshly prepared dilute solution of sodium nitroprusside and 2 ml of strong solution of ammonia. The mixture is shaken and allowed to stand. The presence of acetoacetic acid or acetone is indicated by a light mauve to deep purple colour.

(2) Gerhardt's Ferric Chloride Test A 10 per cent solution of ferric chloride is added to a test tube half full of urine drop by drop until the precipitate of ferric phosphate has reached its full intensity and has wholly or partly redissolved. The solution is then filtered. A few more drops of the ferric chloride solution are added to the filtrate and the presence of ketones is shown by the development of a claret red colour. A similar colour is produced by salicylic acid which may be present in the urine of patients who have taken aspirin or salicylates. The colour due to salicylic acid is not destroyed by boiling whereas acetoacetic acid is changed into acetone by boiling and the colour therefore is destroyed.

Sugar in Blood For determining the amount of sugar in the blood the Folin and Wu and the Hagedorn and Jensen methods are employed.

Before the blood sugar determination is carried out proteins must be removed. In Folin and Wu's colorimetric method a 10 per cent solution of sodium tungstate and dilute sulphuric acid is added to the diluted blood. The tungstic acid formed precipitates the proteins and allows a clear filtrate to be obtained. The filtrate is boiled with an alkaline copper solution and the glucose reduces an equivalent amount of copper to the cuprous state. Cuprous salts produce an intense blue colour with a mixture of phosphoric and molybdic acids which at the same time destroys the blue colour due to unchanged cupric salts. By comparing the blue colour produced with that obtained under similar conditions with standard glucose solutions the amount of glucose in the blood can be determined.

In Hagedorn and Jensen's titration method a protein free filtrate is obtained by adding a measured quantity of blood to a mixture of zinc sulphate and sodium hydroxide in a test tube which is then heated in a boiling water bath for three minutes and filtered. The glucose in the filtrate is determined by boiling with potassium ferricyanide which is reduced by the glucose to potassium ferrocyanide. Potassium ferricyanide liberates iodine quantitatively from potassium iodide in acid

solution. The iodine liberated is determined by titration with sodium thiosulphate solution. Two determinations are made one with ferricyanide which has been boiled with a portion of the blood filtrate and the other with ferricyanide which has been boiled with an equivalent amount of water. The difference in the two readings is proportional to the amount of glucose in the volume of blood filtrate taken.

Pancreas and Other Endocrine Glands. Much of our knowledge of the physiology of the pancreas has been derived from experimental studies of diabetes mellitus in animals. Banting and Best isolated insulin from the pancreas glands of animals and proved its value in the treatment of diabetes mellitus.

In 1924 it was shown that if the anterior lobe of the pituitary gland were removed the animal became more sensitive to insulin and much interest was taken in dogs whose pancreas and pituitary glands had been removed. These experimental dogs, the so-called *Houssay animals*, survived longer than *depancreatised* animals, probably because the removal of the anterior lobe eliminated a hormone acting antagonistically to insulin. Later it was found³⁸ that when extracts of the anterior lobe were injected into dogs or rabbits hyperglycemia and glycosuria developed, suggesting that hyperactivity of the anterior lobe might be the cause of diabetes mellitus. Clinically Atkinson³⁹ has shown that about 30 per cent of patients with *acromegaly*, a disease due to over secretion of the hormones of the anterior lobe, had glycosuria or diabetes mellitus, thus confirming the relation between the anterior lobe and the pancreas. The interrelation between the anterior lobe and the pancreas is further complicated by the fact that according to Marks and Young⁴⁰ an insulin increasing or pancreatrophic substance is also present in the anterior lobe of the pituitary gland.

The adrenal gland has a diabetogenic effect and Ingle^{41, 42} showed that marked hyperglycemia and glycosuria occurred in normal rats fed with a diet rich in carbohydrate when they were injected with large doses of cortisone.

De Finis and Houssay⁴³ demonstrated that a permanent diabetic condition is produced after a few weeks of thyroid feeding in animals whose pancreatic tissue has been partly removed. The blood sugar rises and glycosuria, ketonuria and other signs of diabetes mellitus appear. Discontinuance of thyroid treatment is followed by a rapid return to a normal blood sugar level and recovery of the beta cells in the islets of Langerhans which have not been completely removed. Houssay⁴⁴ states that thyroid treatment can produce diabetes mellitus only in animals with a pancreas reduced by surgical removal or already damaged. It cannot do so in animals with an intact and healthy pancreas. The thyroid gland increases the rate of carbohydrate metabolism and decreases the sensitivity to insulin. John⁴⁵ found that

diabetes mellitus is twice as frequent in patients with hyperthyroidism as in the general population

Ingle⁴⁶ also showed that the artificial oestrogen stilboestrol produced glycosuria even after removal of the anterior lobe of the pituitary gland and the adrenals

Alloxan Diabetes In 1937 Jacobs⁴⁷ showed that the intravenous administration of alloxan an oxidation product of uric acid produced severe hypoglycaemia and convulsions in rabbits and in 1943 Dunn *et al*⁴⁸ showed that the islets of Langerhans in rabbits which have been injected with alloxan are partly or completely destroyed

It is now known that the injection of alloxan is followed by a rise in blood sugar which subsequently falls to convulsant levels if carbohydrates are withheld. The effect can be mitigated by appropriate feeding but twenty four hours after injection the blood sugar level is still low. Further feeding causes the blood sugar level to rise steeply and from this point on the animal is typically diabetic

TABLE II
EFFECT OF ALLOXAN ON BLOOD SUGAR

Time in Hours	0	1	3	5	7	8	9	24	2	26	31	48
Blood sugar mg / 100 ml	100	310	360	115	105		330	90		400	350	600
Treatment	Alloxan 150 mg / kg						Fed		Fed			

Ridout *et al*⁴⁹ showed that alloxan produces temporary hypoglycaemia because it causes atrophy of the islet cells and allows their insulin content to be leached out into the blood stream

Dunn *et al*⁴⁸ discussed the possibility that alloxan may be formed in the body under physiological conditions and suggests that it may be a possible cause of an initial disturbance of the hormone production of the pancreas gland resulting in diabetes mellitus. This work with alloxan is of importance for it shows that a chemical substance can destroy the islets of Langerhans producing all the signs and symptoms of diabetes mellitus in animals but so far little evidence has been obtained that alloxan gives rise to diabetes mellitus in man

Phloridzin Diabetes Phloridzin a substance obtained from the root bark of the apple tree has been used in experimental animals to study carbohydrate metabolism. When it is injected the kidney is no longer able to retain glucose large amounts of which appear in the urine as in diabetes mellitus

Lipocaic In 1924 Allan *et al*⁵⁰ showed that although the glycosuria in depancreatized dogs could be controlled by injections of insulin the animals failed to survive for more than a few months and usually died with an extensive fatty infiltration and degeneration of the liver. If however the dogs received sufficient raw pancreas in the diet insulin kept them alive indefinitely. This curious phenomenon has been investigated by many workers.

Dragstedt *et al*⁵¹ produced an extract of pancreas containing a hormone termed lipocaic which is found in the pancreatic juice and is considered to be essential for the prevention of fatty degeneration of the liver. Best⁵ has pointed out that lecithin and choline can produce the same effect as lipocaic. Successful results have been achieved by the oral administration of lipocaic to a patient with an enlarged liver due to fatty infiltration whereas diabetic children with hepatomegaly have shown marked diminution in the size of the liver after the administration of lipocaic. Dragstedt⁵² suggests that lipocaic may find a place in the control of liver enlargement and possibly arteriosclerosis but this work has not been confirmed and lipocaic is at present more of theoretical than of practical importance.

PITUITARY

The pituitary gland or hypophysis is a small but most important gland which is attached to the under surface of the brain. It is composed essentially of two main lobes—the anterior or glandular portion and the posterior or neural portion—with a small section dividing the lobes called the pars intermedia. The two lobes have different structures and secrete completely different hormones.

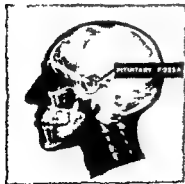


FIG 11

POSITION OF PITUITARY

The gland lies on the under side of the brain at the position marked by the circle.

ANTERIOR LOBE

The anterior lobe of the pituitary has been the subject of considerable research by physiologists and biochemists and many attempts have

been made to isolate the hormones which regulate the activity of other endocrine glands. It is extremely difficult to isolate active fractions in a state of purity and opinion is divided on the number of different hor-



FIG. 12
SECTION OF OVINE PITUITARY

mones produced by the gland. The six given in Table III have each been obtained in preparations almost completely free from other forms of activity and are generally accepted as being well defined separate substances. The existence of ketogenic parathyrotrophic glycotrophic diabetogenic and pancreatrophic substances has also been postulated but it seems likely that their effects are due to one or more of those hormones named in Table III⁵⁴.

TABLE III
HORMONES OF THE ANTERIOR LOBE

Hormone	Function	First Described
Growth Hormone	Necessary for correct skeletal development	Evan and Long 1921
Gonadotrophic Hormones		
(1) Follicle Stimulating Hormone	Controls development of Graafian follicles	Fevold and Hisaw 1933
(2) Luteinizing Hormone	Essential for production of corpora lutea	Fevold and Hisaw 1933
(3) Luteotrophic Hormone	Stimulates milk secretion also concerned with the menstrual cycle and pregnancy	Wallen Larsson 1934
Lactogenic Hormone (Prolactin)		Riddle Bates and Dikslorn 1932
Thyrotrophic Hormone	Essential for correct functioning of the thyroid gland	Loeb 1929 Aron 1939
Adrenocorticotrophic Hormone (Corticotrophin)	Essential for the correct functioning of the adrenal cortex	Smith 1930

A photomicrograph of a section of the anterior lobe which has been stained with eosin and pyrrhol blue shows that three types of cells are present in the gland in varying proportions. They are chromophobe cells which are only faintly stained with aniline dyes and chromophil cells which are divided into acidophil cells and basophil cells according to their staining properties. Acidophil cells are stained by acid dyes such as eosin and basophil cells by basic dyes such as pyrrhol blue.

The chromophobe cells appear to be the mother cells from which basophil and acidophil cells are derived. Severinghaus³⁵ suggests that the specific granules in the different cells are precursors of different hormones. The inactive chromophobe cells can change into hormonally active acidophil or basophil cells and this process can be reversed. The changes in the proportion of each cell type can be interpreted as the result of the need for constant adaptation of the anterior lobe to different requirements of the body. An examination of sections of the anterior lobe in pathological conditions may show an excessive growth of one type of cell for example in acromegaly, a condition due primarily to hyperfunction of the anterior lobe there is hypersecretion of the acidophil cells or eosinophil adenoma of the pituitary. A disease known as Cushing's syndrome is considered to result from a tumour of the basophil cells.

Growth Hormone When the pituitary is removed from young animals growth ceases and Evans and Long⁶ first showed that extracts of the anterior lobe contained growth promoting substances which when injected into rats produced animals of twice the normal size. Highly purified preparations containing the growth hormone have been prepared and Li and Evans³⁷ claim to have isolated the hormone from the gland. It is a water soluble protein which is easily destroyed by many physical and chemical reagents. The growth hormone is essential for correct skeletal development and a deficiency resulting from an underactive pituitary produces dwarfism. The dwarfs differ from cretins whose stunted growth is due to a deficient thyroid gland.

An over production of the growth hormone of the anterior lobe produces persons of stature larger than the average. If the overproduction occurs before the epiphyses of the bones are fully united the person becomes a giant but if it occurs after the bony epiphyses have united the result is acromegaly in which the features are coarse and the bones of the hands and feet are very large in proportion to the rest of the body.

Anterior Lobe and the Gonads The earliest experiments on hypophysectomised animals showed that anterior lobe extracts greatly influence the gonads in males and females and during the last ten to fifteen years much work has been carried out on the gonad stimulating or gonadotrophic factors in the anterior lobe.

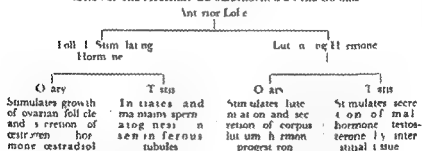
There is an intimate interrelationship between the gonads of both

sexes and the anterior lobe and it has been shown that there is no difference in the hormones derived from the pituitary glands of males and females. Although the existence of as many as eight different hormones capable of stimulating the sex glands has been postulated, more recent work indicates that probably there are only two termed the follicle stimulating hormone (FSH) and the luteinising hormone (LH).

In the female the follicle stimulating hormone stimulates the ovary to produce follicles and secrete the oestrogenic hormone oestradiol. The luteinising hormone stimulates the production of progesterone by the corpora lutea in the ovary after ovulation (see page 44). In the absence of the luteinising hormone luteinisation does not occur in hypophysectomised animals treated with follicle stimulating extracts⁵⁴. These two hormones must be present in the gland in the correctly balanced proportions and they play an essential part in the physiology of the reproductive system.

In the male spermatogenesis and development of the testes are stimulated by the follicle stimulating hormone but it is the luteinising hormone which stimulates the formation of the male sex hormone testosterone which is produced by the interstitial tissue.

TABLE IV
ACTION OF THE PITUITARY GONADOTROPHINS ON THE GONADS



Chorionic Gonadotrophin In 1928 Aschheim⁵⁵ showed that during pregnancy large amounts of gonadotrophic hormones appear in the urine and this is the basis of the best known diagnostic test for pregnancy (see page 46). It was concluded that the source of these hormones was the anterior lobe but further experiments showed that the purified extract from the urine called chorionic gonadotrophin did resemble but was not identical with the luteinising hormone. It has now been shown that the origin of the gonadotrophic factors found in pregnancy urine is the chorionic cells of the placenta.

Serum Gonadotrophin In 1930 Cole and Hart⁵⁶ observed that a follicle stimulating gonadotrophic substance was present in large

amounts in the blood of pregnant mares reaching a maximum concentration on about the seventieth day of gestation. Boycott and Rowlands⁶¹ investigated the biological nature of the extracts of pregnant women's serum and made the interesting observation that in contrast to the mares serum extract it possessed only luteinising properties similar to those of the extracts which had been prepared from pregnancy urine.

Both chorionic gonadotrophin and serum gonadotrophin are available commercially. In general, chorionic extracts comprise mainly the luteinising hormone while serum gonadotrophin is mainly the follicle stimulating hormone.



FIG. 13

ACTION OF SERUM GONADOTROPHIN

Ovaries and uterus of immature rats before and after treatment with serum gonadotrophin

The synergistic action of the luteinising chorionic gonadotrophin from pregnancy urine and an extract containing the follicle stimulating hormone of the anterior lobe has been investigated. Evans, Meyer and Simpson⁶² observed that a combination of the two hormones produced a greater increase in ovarian size and weight than could be obtained by administering the components separately. A standardised product is available and several reports indicate that it has a place in hormone therapy.^{63 64 65}

Anterior Lobe and Other Glands It has been shown by many workers that a close interrelation exists between the anterior lobe of the pituitary and other glands including the thyroid, adrenals, mammary glands and pancreas. Purified extracts of the anterior lobe containing the thyrotrophic hormone induce hyperplasia of the thyroid and stimulate the liberation of an abnormal amount of thyroxine.⁶⁶ The effects of their administration to experimental animals have been investigated^{67 68 69}, but few clinical reports are available. Marx *et al.*⁷⁰ suggest that the thyrotrophic hormone acts synergistically with the

growth hormone and consider that it may be of value in the treatment of pituitary dwarfism

Extracts from the anterior lobe produce hypertrophy of the adrenal cortex. The active principle called the adrenocorticotrophic or corticotrophic hormone (ACTH) has been obtained in a pure form⁷¹ and shown to control the activity of the adrenal cortex (see page 38)

The existence of a specific hormone in the anterior lobe which influences the growth of the mammary gland during pregnancy and is responsible for the secretion of milk has been confirmed and is known as the lactogenic hormone or prolactin⁷²⁻⁷³. It is also known as the luteotrophic hormone because it assists in maintaining the corpus luteum produced by the action of the luteinising hormone

POSTERIOR LOBE

Extracts of the posterior lobe of the pituitary can be separated into two fractions: one containing an oxytocic principle which causes a contraction of the uterus (the word oxytocic is derived from Greek words meaning rapid childbirth) and one consisting mainly of a substance which causes a rise in blood pressure under certain conditions and which has also an important antidiuretic effect reducing the amount of urine excreted. The former principle is called oxytocin and the latter vasopressin.

Verney⁷⁴ showed in experiments on the isolated kidney which is perfused with blood that the urine excreted was very dilute and resembled that obtained from a patient with diabetes insipidus. When an extract of the posterior lobe was added to the perfusion fluid the kidney recovered its power to excrete a concentrated urine. Verney also showed that the polyuria following isolation of the mammalian kidney was due to the removal of this organ from the normal sustained antidiuretic action of the pressor fraction of the posterior lobe.

Injection of extracts of the posterior lobe produces a direct action on the capillaries which contract causing pallor of the skin. The action of the extracts on the blood pressure varies with different animals. Generally there is no significant rise in blood pressure; there may be a momentary fall succeeded by a more prolonged increase in the pulse rate. Extracts of the posterior lobe have been used to raise the blood pressure after shock. If the posterior lobe of the pituitary is damaged by disease or injury the hypothalamic syndrome may develop when the individual becomes exceptionally sleepy and emotional control is upset. The normal water balance is impaired because the renal tubules are no longer able to reabsorb water from the urine and to make up for the large volume of water lost; equally large volumes must be imbibed.

Pars Intermedia. The posterior lobe of the pituitary gland includes the pars intermedia, the small portion of the gland dividing

the two lobes. In man its function is unknown but it is well developed in fishes, amphibians and reptiles. Frogs can change their colour to make themselves less conspicuous and this is due to an alteration in the pigment cells which are stimulated by the hormone of the pars intermedia. The pigment cells, melanophores or black cells, are expanded and dispersed.

ADRENALS

The adrenal or suprarenal glands are situated on the upper surface of the kidneys and are composed of two different tissues: an outer layer or cortex and an inner part or medulla.



FIG. 14

POSITION OF ADRENAL

The adrenal (A) lies on the upper surface of the kidney (B). The photograph also shows the connecting nerve plexus (C) and the aorta (D).

The adrenal glands have a particularly rich blood supply which is an indication of the importance of these organs and they secrete groups of hormones with widely differing actions.

Adrenal Medulla In 1894 Oliver and Schafer⁷⁶ showed that injection of an extract of the adrenal medulla produced a remarkable rise in blood pressure and later workers succeeded in isolating from the gland a crystalline compound which they called epinephrine. The active principle obtained in pure form in 1901 by Takamine⁷⁸ and others was marketed as Adrenalin which is a Latin rendering of the Greek term epinephrine and it is now known as adrenaline except in

the United States of America where it is still called epinephrine. Adrenaline was the first hormone to be isolated in crystalline form.

Unlike the cortex the medulla is apparently not essential to life and in spite of extensive studies on its physiological function its action in persons under normal conditions is unknown. It has been suggested that adrenaline acts as an emergency hormone because it is secreted in increased amounts in times of physical or emotional stress and enables the body sometimes to perform seemingly impossible tasks. This theory has not been substantiated by experiments on adrenalectomised animals but it has been shown that the barking of a dog increases the rate of a cat's denervated heart.



FIG. 15

SECTION OF ADRENAL

From the fibrous capsule numerous trabeculae containing blood sinuses run at right angles into the white fibrous and yellow fatty tissues of the cortex dividing it into columns of cells. The dark central portion the medulla is red and richly supplied with blood.

Action of Adrenaline Adrenaline is a sympathomimetic drug that is its effects in the body are comparable to those produced by stimulating the sympathetic nervous system. Other compounds chemically related to adrenaline have been investigated by Barger and Dale⁷⁷ and the pharmacological action and therapeutic uses of related compounds are described by Gunn⁷⁸. Slight changes in the chemical constitution may produce considerable variations in pharmacological activity.

Adrenaline on injection produces many complex physiological effects in the body and has many therapeutic uses. Its action is immediate and its effect transient. Perhaps its most important action is the

raising of the blood pressure adrenaline is the most potent vasopressor drug known and if given intravenously or directly into the heart muscle it produces a sudden rise of blood pressure lasting for a few minutes due mainly to its constricting effect on the blood vessels

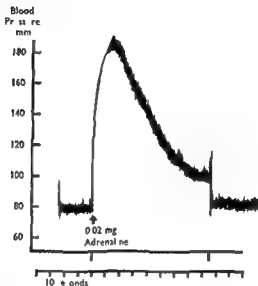


FIG 16

ACTION OF ADRENALINE

Graph showing the rapid rise in blood pressure after injecting adrenaline into a rabbit

Adrenaline relaxes the bronchial musculature and is therefore used extensively in the prophylaxis and treatment of asthmatic attacks. In addition it dilates the pupils of the eye and inhibits movement in the uterus, intestines and bladder. Adrenaline plays an important part in carbohydrate metabolism; it stimulates the conversion of liver glycogen to blood sugar and can be considered to have an antagonistic action to insulin. Cori and Cori⁹ consider that adrenaline accelerates the cycle of muscle glycogen to liver glycogen while insulin accelerates that of blood glucose to muscle glycogen, leading to hypoglycaemia and depletion of liver glycogen.

Action of Noradrenaline Noradrenaline, which differs from adrenaline by the absence of a methyl group from the nitrogen atom, was first synthesised by Stolz¹⁰ in 1904, but its presence in the adrenal gland was not reported until 1949 when Holton¹¹ showed that tumours of the medulla contained large amounts of noradrenaline. The presence of noradrenaline in the adrenal medulla, in addition to adrenaline, has

now been established⁸³ and pharmacological and clinical studies have shown that noradrenaline exerts a more powerful effect on the heart than adrenaline. It is believed that noradrenaline is a precursor of adrenaline and that essential hypertension results from failure of the body to effect methylation. Noradrenaline has been shown to be effective in the treatment of certain cases of acute hypotension⁸⁴.

Adrenal Cortex: The cortex or outer layer of the adrenal gland is essential to life and the effects of adrenal cortical deficiency, a clinical example of which is Addison's disease, are marked disturbances in the metabolism of electrolytes, carbohydrates, proteins and fats associated with loss of appetite, general muscular weakness, decreased resistance to cold, low blood pressure, loss of weight and vomiting. In man there is pigmentation of the skin, chiefly on exposed areas, but this does not occur in experimental animals. Some of these symptoms can be controlled by the administration of sodium chloride by mouth or by the injection of normal saline solution and a diminished intake of potassium salts, but replacement therapy with adrenal cortical extracts or synthetic deoxycortone acetate, which does not have exactly the same action as the natural extract, is usually employed. Overactivity of the adrenal cortex, frequently due to a tumour, leads to a condition known as adrenal virilism, which is characterised by precocious growth and sexual maturity; in females there is masculinisation.

An important function of the adrenal cortex is the maintenance of correct electrolytic balance or relative concentration of sodium and potassium salts in the body. An underactive gland gives rise to an increased excretion of sodium followed by a fall in the serum chloride and bicarbonate, accompanied by a rise in the concentration of potassium in the extracellular fluids and its retention in the body cells. It is possible to prolong the lives of adrenalectomised animals by the administration of sodium chloride alone. The adrenal cortex also affects water metabolism. After adrenalectomy there is a reduction in the diuretic response to water and a tendency to water intoxication. The administration of adrenal cortical extracts in man leads to profuse diuresis.

Twenty-eight crystalline steroids have been isolated from the cortex. In 1934 Kendall *et al.*⁸⁵ isolated a crystalline substance capable of maintaining the life of adrenalectomised animals. In 1936 Reichstein⁸⁶ obtained from the adrenal cortex a sterol with androgenic properties which he named *androsterone*. Ingel⁸⁷ isolated seven compounds which were effective in relieving different conditions associated with adrenal cortical deficiency, one of the most active being deoxycortone.

Adrenal Cortex and Other Glands: The hormones of the adrenal cortex and the gonads are closely related chemically (see page 77) and adrenal cortical insufficiency profoundly affects the

secondary sex characteristics Speer⁸⁸, by injection of deoxycortone acetate has produced changes in the ovary similar to those produced by the ovarian hormones. Other workers have separated oestrogenic and progestational substances from adrenal cortical extracts.

Many relationships between the adrenals and the other endocrine glands have been postulated and although a considerable amount of experimental work has been carried out it is not yet clear to what extent the glands are physiologically related. It is known that the cortex is concerned with carbohydrate metabolism (see page 26). The adrenocorticotrophic hormone of the anterior lobe of the pituitary stimulates the adrenal cortex and conversely adrenal cortical deficiency may be accompanied by dysfunction of the anterior lobe of the pituitary. In some syndromes for example Cushing's syndrome there may be either an adrenocortical or an anterior pituitary tumour.

There are some factors common to both the adrenals and the gonads. The administration of oestrogenic compounds causes hypertrophy of the adrenals. Androgens on the other hand either have no effect on the size of the adrenals or produce atrophy of the glands. Castration in the male animal is followed by adrenal cortical hypertrophy.

The work of Selye⁸⁹ shows that the adrenal cortex plays an important part in the protection of an animal from environmental stress. He has further shown that this process of adaptation to stress may be accompanied in man by pathological conditions which he calls diseases of adaptation and which include rheumatoid arthritis and hypertension.

On this theoretical basis research was carried out in Germany during the Second World War on the administration of adrenal cortical hormones to air crews as a means of protection from the great strain of high altitude flying. Of even greater medical importance is the discovery by Hench and his colleagues⁹⁰ that cortisone administered in a dosage of 100 milligrams a day will rapidly alleviate the symptoms of rheumatoid arthritis and rheumatic fever. The adrenocorticotrophic hormone of the anterior lobe of the pituitary has been shown to give similar beneficial results in the rheumatic diseases.

GONADS

The gonads or sex glands comprise the ovaries in the female and the testes in the male and they produce hormones which greatly influence the body as a whole. Their main function is concerned with reproduction but they are also determining factors in the personal make up both physical and psychological.

Before discussing the hormones secreted by the gonads consideration should be given to the anatomy of the male and female reproductive systems. In the embryo during the early weeks the gonad is morphologically a bisexual organ which may develop into either an ovary or a testis depending on the predominant hereditary sex characteristics.

Male Reproductive System The male reproductive system consists of the testes the prostate gland lying below the bladder the seminal vesicles and Cowper's glands the secretions of which are added to the spermatozoa. The testes contain convoluted seminiferous tubules which produce spermatozoa and in between these tubules there are groups of interstitial cells the source of the male hormone testosterone.

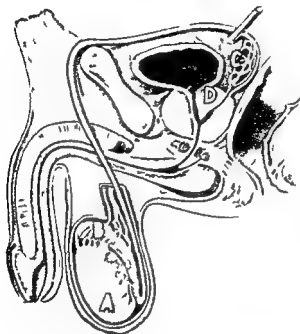


FIG. 17

MALE SEX ORGANS

(A) Testis (B) Epididymis (C) Seminal vesicle (D) Prostate

Spermatozoa are transferred from the seminiferous tubules to the epididymis an exceedingly tortuous tubule about 6 metres in length on the posterior surface of the testis. Here they are stored and mature. The production of spermatozoa and testosterone usually continues throughout life and there is so far as is known no periodic alteration in the amount of hormone secreted analogous to the menstrual cycle in the female. There is probably a diminution in older men a male climacteric having been described which may be relieved by replacement therapy. An extract from bulls' testes when injected into cacons produces growth of the comb wattles and ear lobes. The effect of

injections of different androgens and also of oestrogens on capons has been the subject of many investigations and it is a convenient test for assessing the potency of different hormone preparations

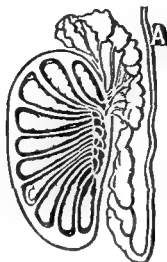


FIG. 11

TESTIS

Diagram showing the relation between the vas deferens (A) the epididymis (B) and the convoluted tubules of the testis (C)



FIG. 12

EFFECT OF TESTOSTERONE ON
CAPON'S COMB

Left before administration of testosterone
right after administration

The effects of castration have been known for centuries. There is a profound alteration in somatic growth: the bones become longer, the muscles are less developed, and fat is deposited within the muscles. When castration in the human male is performed before puberty, the high pitched voice is retained, and the boy does not develop typical

male characteristics such as the normal distribution of hair. If it is performed after puberty the effects are less marked and secondary male characteristics can be restored by the injection of testosterone.

The testes are closely related physiologically to the anterior lobe of the pituitary gland (see page 30) and if the pituitary gonadotrophic hormones are imperfectly secreted development of the testes is retarded and their function diminished.

Perhaps the most typical sex characteristic which is influenced by the male hormone is the distribution of hair and it is interesting to note that the pattern of distribution of head hair in men and women may be determined by the hormones produced by the gonads. Hamilton¹¹ suggests that baldness is an hereditary trait requiring a physiological level of androgen for its development. It is not produced by androgens in individuals not carrying the necessary hereditary factors. The colour of the skin, distribution of subcutaneous fat, depth of the voice, skeletal growth and greater muscular strength of the male are secondary sex characteristics resulting from the action of the male hormone.

Female Reproductive System · The essential organs of reproduction in the female are the two ovaries, ovoid organs each weighing about 5 grammes. They are suspended in the pelvis and are in contact with the uterine or fallopian tubes which are about 10 centimetres long. The ovaries during the period of reproductive life alter rhythmically

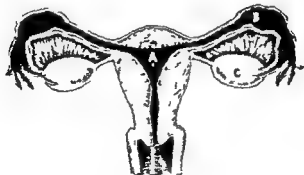


FIG. 20

THE FEMALE SEX ORGANS

(A) Uterus (B) Fallopian tube (C) Ovary

cally in structure and perform a dual function: the production of ova and of two hormones, oestradiol and progesterone, which are necessary for the functioning of the female cycle and for the maintenance of pregnancy.

At birth the ovaries contain from 30 000 to 400 000 ova. The number of ova progressively diminishes throughout life from 400 to 500 attaining maturity usually one at monthly intervals. Each ovum is carried through the fallopian tube to the uterus. If fertilisation takes place it occurs in the fallopian tube and the fertilised ovum is then transferred to the uterus. If fertilisation does not occur the ovum degenerates and is expelled during menstruation.

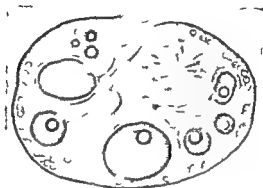


FIG. 21

SECTION OF OVARY ($\times 2$)

The ovary consists of a mass of stroma covered by germinal epithelium which contains primordial ova. Graafian follicles enclosing ova at various stages of maturation, corpora lutea, blood vessels, lymphatics and nerves. One follicle (*top left*) has burst and expelled its ovum. The larger non-nucleated mass of an old follicle (*top right*) has developed into a corpus luteum.

The uterus or womb is a thick-walled muscular organ with an internal mucous lining (endometrium), a muscular coat (myometrium) and an external peritoneal covering. The lower end of the uterus becomes narrower and leads to the vagina, the passage from the uterus to the exterior of the body. If an ovum is fertilised it becomes embedded in the wall of the uterus and is normally retained there until pre-natal development is completed.

Reproductive Cycle in Mammals. At maturity the female reproductive system in mammals undergoes regular periodic changes. In most mammals it is usual to divide the cycle into four stages —

- pro-œstrus when the follicle ripens
- œstrus the period of heat when ovulation occurs
- metœstrus the period when retrogressive changes occur in the ovary
- diœstrus or anœstrus the period of sexual inactivity

In 1917 Stockard⁹ demonstrated that there were characteristic changes in the cells of the vaginal epithelium of animals during the œstrus cycle. These changes can be followed by a microscopic examination of vaginal smears taken at different phases of the cycle which forms the basis of much experimental investigation of the ovarian hormones and their artificial analogues (see page 133).

During diœstrus vaginal smears consist mainly of leucocytes with few nucleated cells. During pro-œstrus leucocytes are absent and a large number of nucleated epithelial cells are present. During œstrus the epithelial cells lose their nuclei and non nucleated cornified cells are found in large numbers. Vaginal smears taken during metœstrus show many leucocytes with some epithelial cells.

Human Reproductive or Menstrual Cycle The human female reproductive cycle or menstrual cycle is different from the œstrus cycle in lower mammals. It is however dependent on the production by the ovary of the correct amounts of hormones at regular times and also on the correct balance between the gonadotrophic hormones of the anterior lobe of the pituitary and the ovarian hormones. The cycle in humans normally occupies twenty-eight days and recurs regularly from puberty until the menopause when declining reproductive activity finally ceases.

Changes in the Uterus As the menstrual cycle is a continuous building up, shedding and repairing of the endometrial lining, there is no clear cut division between one stage of the process and the next. The cycle may however conveniently be divided into three main phases —

(1) A bleeding phase or period of menstruation characterised by a degeneration of the endometrium and occupying approximately four days.

(2) A proliferative or pre-ovulatory phase lasting seven to ten days in the later stages of which the epithelium of the uterine mucosa develops and increases in thickness, the cells at the surface of the epithelium becoming more columnar. From the fourth to the eighth day of the cycle the tissue is resting and recovering from the effects of menstruation. From the eighth to the thirteenth day, under the stimulation of the œstrogenic hormone, regeneration occurs. On the basal layer of the inner surface of the uterus a gradually thickening layer of glandular tissue, the endometrium, develops reaching its maximum thickness by about the fifteenth day. This layer consists of straight tubular, initially inactive glands separated by connective tissue. At about the fourteenth day a Graafian follicle in the ovary ruptures and liberates an ovum which may or may not later be fertilised.

(3) A progestational or post-ovulatory phase lasting for approximately fourteen days after ovulation when the glands undergo characteristic changes becoming twisted and spiral instead of straight.

The cells lining the glands begin to elaborate glycogen and the connective tissue changes to form characteristic polyhedral decidual cells. During this phase the endometrium is highly vascular and is ready to receive and implant a fertilised ovum which if present, becomes embedded in the vascular endometrial tissue. A placenta is ultimately built up and the ovarian membranes develop round the growing foetus. If however the liberated ovum is not fertilised the uterine mucosa again slowly degenerates and menstruation occurs. The period occupied by each phase of the cycle as described above is only an average. In practice considerable variations occur in the timing of the cycles of different women and even of the same woman.

Changes in the Ovaries Concurrently with these changes in the uterus cyclic changes are taking place in the ovaries. A primordial follicle of one of the ovaries becomes distended with fluid (liquor folliculi). The swollen follicle on attaining maturity bursts at the surface of the ovary and the ovum passes into the fallopian tube. After the discharge of the ovum changes occur within the ruptured ovarian follicle resulting in the formation of a glandular structure the cells of which become filled with a yellow fat lutein from which the name corpus luteum (yellow body) is derived and the hormone progesterone is secreted. Progesterone is essential for —

- (a) The development of a premenstrual endometrium
- (b) Maintenance of pregnancy
- (c) Growth of the lobules of the breast
- (d) Inhibition of ovulation during pregnancy

Action of the Anterior Lobe of the Pituitary The cyclic changes in the ovary resulting in the maturation of an ovum which is discharged and the growth of the corpus luteum in the ruptured follicle are controlled by the gonadotrophic hormones of the anterior lobe of the pituitary gland. Thus the follicle stimulating hormone stimulates the production of the ovarian hormone oestradiol which is secreted in increasing amounts from the fourth to the twenty eighth day of the cycle and the luteinising hormone stimulates the production of the corpus luteum hormone progesterone which is secreted in increasing amounts from the fourteenth to the twenty eighth day of the cycle.

There is some doubt as to how the two ovarian hormones and the two pituitary gonadotrophic hormones act in the establishment of the menstrual cycle but it is known that a definite balance exists and must be maintained between the hormones elaborated by the different glands. The amount of oestrogens and progesterone present in the ovaries cannot be measured directly but an estimation of the excretion products is of importance in diagnosis and treatment.

Excretion of Progesterone In the normal menstrual cycle progesterone is eliminated in the urine as an inactive water soluble compound

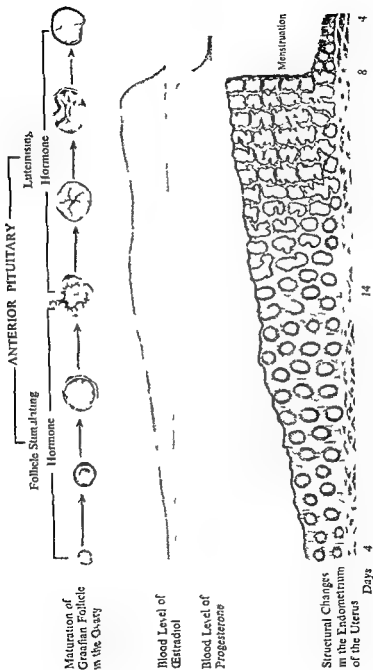


FIG 22

DIAGRAMMATIC REPRESENTATION OF NORMAL MENSTRUAL CYCLE

sodium pregnanediol glycuronide and the excretion of this pregnanediol closely parallels the production of progesterone by the corpus luteum. Excretion begins about twelve days before the onset of menstruation (a day or two after ovulation) and reaches a peak about

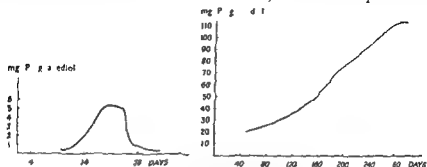


FIG. 23

PREGNANEDIOL EXCRETION

The left hand chart shows the daily excretion during the menstrual cycle. The right hand chart shows the very much larger daily excretion during pregnancy.

one week before menstruation. The maximal daily excretion is approximately 5 milligrams. During pregnancy, however, the output of pregnanediol increases considerably from the twentieth to the ninetieth day and may reach 60 to 100 milligrams daily by the ninth month. Probably most of the progesterone produced after the third month of pregnancy is made by the placenta and not the corpus luteum, and failure of the placenta to produce progesterone may be regarded as one of the reasons for abortion.

PREGNANCY DIAGNOSIS

During pregnancy, oestrogens, progesterone, and the gonadotrophic hormones of the anterior lobe of the pituitary gland are excreted in increased amounts, and the presence of the chorionic gonadotrophic hormone in the urine forms the basis of biological tests for the existence of pregnancy.

Aschheim Zondek Test. In the Aschheim Zondek test²³ 0.5 millilitre of a morning sample of urine is injected subcutaneously twice daily for three consecutive days into each of six immature female white mice 3 to 4 weeks old and weighing 6 to 8 grammes. The animals are killed 100 hours after the first injection and their ovaries examined. If the ovaries are larger than normal and have large follicles and corpora lutea, some of which may show haemorrhagic spots (blood points), the test is positive.

Friedman
Aschheim

t A rapid and reliable modification of the
t was made by Friedman^{24, 25} who discovered

that a single intravenous injection of 1 to 15 millilitres of pregnancy urine usually induced ovulation within forty eight hours in young mature non pregnant female rabbits. Unlike most other animals the rabbit does not ovulate spontaneously and the test is positive when the ovaries at autopsy show the presence of recently ruptured follicles with haemorrhagic areas.

Xenopus Test In 1930 Hogben⁹⁶ showed that extracts of the anterior lobe of the pituitary induced ovulation in the hypophysectomised mature South African claw toed frog (*Xenopus laevis*) and this fact was subsequently made use of in developing the xenopus pregnancy test sometimes referred to as Hogben's test.

The mature female xenopus carries eggs throughout the year but extrudes them only after mating or under laboratory conditions after the injection of chorionic gonadotrophin. This test is of interest in that it is comparatively simple to carry out, no operation is required and the result is known in from four to eighteen hours. The frogs are kept in tanks of water at 70 F and are fed on liver, beef heart and earthworms. 1 millilitre of an extract of the urine is injected subcutaneously into the dorsal lymph sac of the frog which is returned to the tank and observed at intervals after four hours for the extrusion of eggs. If the test is positive eggs usually appear in from six to twelve hours; if none appears at the end of eighteen hours the test is negative. Reliable results can be expected in 96 to 99 per cent of cases and are first demonstrable about ten days after the first missed menstruation.

Male Toad Tests Galli Mainini⁹⁷ used the male toad *Bufo arenarum* as the test animal. Usually 10 millilitres of the patient's urine is injected subcutaneously and after a few hours a drop of urine is collected from the toad and examined for the presence of spermatozoa. Good results have also been obtained using English male toads (*Bufo vulgaris*)⁹⁸ several South American toads and the frog *Rana pipiens*.⁹⁹

In all these tests false positives may be obtained in patients with hydatidiform mole and chorionepithelioma but false negatives rarely or never occur. Hydatidiform mole occurs when the ovum fails to develop and there is abnormal growth of the tissues concerned in the formation of the membranes which normally surround the developing embryo. Chorionepithelioma is a form of malignant disease arising from the placental relics of a former pregnancy or of a hydatidiform mole.

Guterman Test This test¹⁰⁰ consists essentially of the extraction with toluene of the acid hydrolysed pregnanediol complex of urine followed by the purification and precipitation of the pregnanediol. Ten millilitres of concentrated sulphuric acid is added to the precipitate from 100 millilitres of a morning specimen of urine and the colour

is observed in a test tube when solution is complete. Colourless to light yellow is taken as negative deep yellow to deep orange brown as positive

Conditions which are associated with the excretion of luteinising hormone such as hydatidiform moles and testicular tumours give negative results by this technique which therefore has the advantage of distinguishing between normal placental and abnormal placental or placental like tissue

REFERENCES

- 1 ZONDER H Diseases of the Endocrine Glands Translated by C P GILES 2nd English Edition 1944 Arnold London
- 2 WEGELIN C and ABELIN I *4th exp Path* 1921 89 219 1925 105 137
- 3 ROBERTSON J D *Brit med J* 1944 1 617
- 4 GORDON E H and ALBRIGHT E C *J Amer med Ass* 1950 143 1129
- 5 RIVERS R P and RANDALL H S *J Endocinol* 1945 4 221
- 6 HEINEKE E P and TURNER C W *J biol Chem* 1943 149 555
- 7 FARNES A S *J Endocinol* 1946 4 476
- 8 CHESNEY A M CLAWSON T A and WEBSTER B *Johns Hopk Hosp Bull* 1928 43 261
- 9 MACKENZIE J B MACKENZIE C G and MCCOLLUM E V *Science* 1941 94 518
- 10 MACKENZIE C G and MACKENZIE J B *Endocrinology* 1943 32 185
- 11 ASTWOOD E B *et al Endocrinology* 1943 32 210
- 12 ASTWOOD E B *J Amer med Ass* 1943 122 78
- 13 LEYS D *Lancet* 1945 1 461
- 14 WILSON V *Lancet* 1946 1 640
- 15 ASTWOOD E B BISSELL V and HUGHES A M *Endocrinology* 1945 37 456
- 16 ANDERSON G W *et al J Amer chem Soc* 1945 67 2197
- 17 BAVIN E M and GOODCHILD D A *Nature Lond* 1946 157 659
- 18 ASTWOOD E B and VANDER LAAN W P *J clin Endocrinol* 1945 5 424
- 19 ALBRIGHT F and REIFENSTEIN L C The Parathyroid Glands and Metabolic Bone Disease 1948 Baillière Tindall and Cox London
- 20 NEUFELD A H and COLLIP J H *Endocrinology* 1947 30 135
- 21 ALBRIGHT F *J Amer med Ass* 1939 112 2592
- 22 ROBERTSON J D *Lancet* 1941 11 795
- 23 SHELLING D H *J biol Chem* 1931 96 215
- 24 HOLTZ F *J clin Endocrinol* 1941 1 453
- 25 SEVERINGHAUS E L and ST JOHN R *J clin Endocrinol* 1943 3 635
- 26 BRACK W *Schweiz med Wschr* 1946 76 316
- 27 LAWRENCE R D and McCANCE R A *Brit med J* 1934 1 981
- 28 HAGEDORN H C *et al J Amer med Ass* 1936 106 177
- 29 HAGEDORN H C *Proc R Soc Med* 1937 30 805
- 30 SCOTT D A and FISHER A M *J Pharmacol* 1935 55 206
- 31 SCOTT D A and FISHER A M *J biol Chem* 1936 114 1555
- 32 SCOTT D A and FISHER A M *J Pharmacol* 1936 58 78
- 33 BAVIN E M and BROOM W A *Quart J Pharm* 1937 10 327
- 34 BROOM W A and BAVIN E M *Quart J Pharm* 1937 10 334
- 35 BISCHOFF F *Amer J Physiol* 1936 116 239
- 36 GRAY P A *Endocrinology* 1936 20 461
- 37 REINER L SEARLE D S and LANG E H *Proc Soc exp Biol NY* 1939 40 171
- 38 EVANS H M *et al Proc Soc exp Biol NY* 1932 29 857
- 39 ATKINSON F R H *Endocrinology* 1938 20 245
- 40 MARKS H F and YOUNG F G *Lancet* 1940 1 493
- 41 INGLE D J *Endocrinology* 1941 29 649
- 42 INGLE D J *Endocrinology* 1941 29 838
- 43 DE FRIES M L and HOUSSAY B A *Rev Soc argent Biol* 1944 19 94
- 44 HOUSSAY B A *Clin Proc* 1946 5 219
- 45 JOHN H J *J clin Endocrinol* 1942 2 264

- 46 INGLE D J *Endocrinology* 1944 34 361
- 47 JACOBS H H *Proc Soc exp Biol NY* 1937 37 497
- 48 DUNN J S SHEPHERD H L and McLEITCH N G B *Lancet* 1943 i 484
- 49 RIDOUT J H HAM A W and WRENSHALL G A *Science* 1944 100 57
- 50 ALLAN I N *et al Brit J exp Path* 1974 5 75
- 51 DRAGSTEDT L I *et al Amer J Physiol* 1936 117 175
- 52 BEST C H *Diabetes and Insulin and the Lipotropic Factors* 1949 Thomas Springfield Illinois
- 53 DRAGSTEDT L R *J Amer med Ass* 1940 114 29
- 54 GAUDUM J H and LORAIN J A *J Pharm Pharmacol* 1950 2 65
- 55 SEVERINGHAUS A E *Physiol Rev* 1937 17 556
- 56 EVANS H M and LONG J A *Inat Rec* 1921 21 62
- 57 LI C H and EVANS H M *Science* 1941 99 183
- 58 WITSCHI E *Endocrinology* 1940 27 437
- 59 ASCHHEIM S *Flin Hschr* 1928 7 1453
- 60 COLE H H and HART G H *Amer J Physiol* 1930 93 57
- 61 BOYCOTT M and ROWLANDS I W *Brit med J* 1939 1097
- 62 EVANS H M MEYER K and SIMPSON M E *Proc Soc exp Biol NY* 1931 28 845
- 63 ROBERTS C L *J Obstet Gynec* 1946 53 149
- 64 MAZER C and KAVETZ E *Amer J Obstet Gynec* 1941 41 474
- 65 DAVIS A *J Obstet Gynec* 1944 51 401
- 66 FRAENKEL CONRAT J FRAENKEL CONRAT H SIMPSON M E and EVANS H M *J Biol Chem* 1940 135 199
- 67 SIEBERT W J and SMITH R S *Amer J Physiol* 1930 95 396
- 68 FRIEDGOOD H B *Johs Hopk Hosp Bull* 1934 54 49
- 69 WILKINS L and FLEISCHMANN W *J Amer med Ass* 1941 116 2459
- 70 MARY W SIMPSON M E and EVANS H M *Proc Soc exp Biol NY* 1942 49 594
- 71 LI C H SIMPSON M E and EVANS H M *Science* 1942 96 450
- 72 WHITE A CATCHPOLE H R and LONG C N H *Science* 1937 86 82
- 73 LI C H SIMPSON M E and EVANS H M *J Biol Chem* 1942 146 627
- 74 VERNEY F B *Lancet* 1929 i 539
- 75 OLIVER G and SCHAFER F A *J Physiol* 1894 16 i
- 76 TAKAMINE J *Amer J Pharm* 1901 5 573
- 77 BARGER G and DALE H H *J Physiol* 1910 41 19
- 78 GUNN J A *Brit med J* 1939 ii 155 and 214
- 79 CORI C F and CORI G T *J Biol Chem* 1929 81 391
- 80 STOLZ F *Ber Disch chem Ges* 1904 37 4149
- 81 HOLTON P *Nature Lond* 1949 163 210
- 82 GOLDFENBERG M FABER M ALSTON F J and CHARNOFF F C *Science* 1940 109 534
- 83 RULBRING E and BURN J H *Brit J Pharm* 1949 4 202
- 84 GOLDENBERG M APPAR V DETERLING R and PINES H I *J Amer med Ass* 1949 140 776
- 85 KENDALL F C *et al Proc Mayo Clin* 1934 9 245
- 86 REICHSTEIN T *Helv chim Acta* 1936 19 273
- 87 INGLE D J *Endocrinology* 1942 31 419
- 88 SPEERT H *Johs Hopk Hosp Bull* 1940 67 189
- 89 SELYE H *Phactones* 1949 163 393
- 90 HENCH P S KENDALL F C SLOCUM C H and POLLEY H P *Proc Mayo Clin* 1949 24 181
- 91 HAMILTON J B *Amer J Anat* 1942 71 451
- 92 STOCKARD C R *Amer J Anat* 1917 22 225
- 93 ASCHHEIM S and ZONDER B *Kl Wschr* 1928 7 831 and 1404
- 94 FRIEDMAN M H *Amer J Physiol* 1929 90 617
- 95 FRIEDMAN M H and LAPIER M F *Amer J Obstet Gynec* 1931 21 405
- 96 HOGREN L J *Proc Soc Sci Series A* 1930 5 19
- 97 GALLI MAININI C *J Amer med Ass* 1918 138 121
- 98 KLOPPER A and FRANK H *Lancet* 1949 ii 9
- 99 WITBERGER P B and MILLER D F *Science* 1948 107 198
- 100 GUTERMAN H S *J Clin Endocrinol* 1944 4 762

CHAPTER III

CHEMISTRY OF THE NON STEROID HORMONES

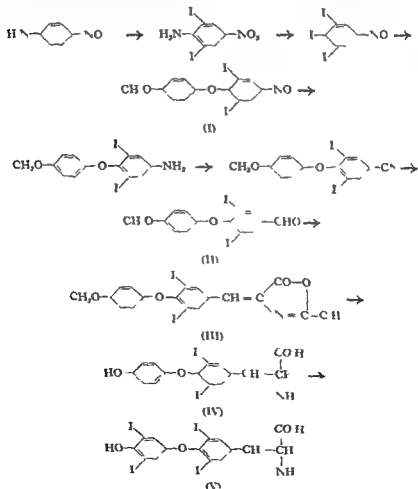
THE non steroid hormones comprise those which are derived from the thyroid parathyroids pancreas pituitary (both posterior and anterior lobes) and adrenal medulla. The hormones associated with the parathyroids pancreas and pituitary possess the chemical and physical characteristics of proteins and with the exception of insulin little is known of the details of their chemical constitution. On the other hand the hormone of the thyroid thyroxine and those of the adrenal medulla adrenaline and noradrenaline are relatively simple chemical compounds of known constitution.

THYROID

The isolation of the active principle from the thyroid gland was first achieved by Kendall¹ but the method of isolation was subsequently modified and improved by Harington². Since the hormone is present in the form of a peptide in the protein iodothyroglobulin hydrolysis has to be carried out firstly with aqueous sodium hydroxide and subsequently with aqueous baryta. In Harington's method hydrolysis is carried out with hot baryta using successively a 10 per cent solution and a 40 per cent solution. In this manner the hormone is isolated as the barium salt which may be decomposed by addition of a slight excess of aqueous sodium sulphate to its suspension in hot 1 per cent aqueous sodium hydroxide. After filtration from barium sulphate the free thyroxine may be isolated by acidification. Subsequent purification is effected either by dissolving the crude product in alcoholic alkali followed by acidification with acetic acid or by crystallisation of the sodium salt. Thyroxine obtained by this method is optically inactive but by utilising a process of enzymic hydrolysis Harington later obtained L thyroxine m.p. 235° [α]_D²⁵ - 3.8 (sodium salt in 60 per cent ethyl alcohol)⁴.

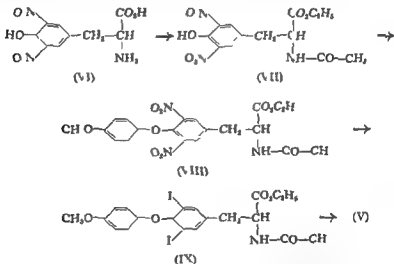
Thyroxine ($C_{15}H_{11}O_4NI_4$) Thyroxine (V) is a white crystalline compound which is sparingly soluble in water and insoluble in organic solvents. It dissolves in aqueous ammonia and in alcoholic alkalis. The constitution of thyroxine was established by Harington³ who was able to replace the iodine in the molecule by hydrogen by shaking an alkaline solution of the hormone with colloidal palladium in the presence of hydrogen. The resulting compound $C_{15}H_{15}O_4N$ known as thyronine was shown to be an amino acid and its constitution was proved by synthesis. Finally the synthesis of thyroxine itself was reported by Harington and Barger in 1927⁴. In this synthesis *p*-nitraniline was converted into 2,6-diiodo-4-nitroaniline and then by means of the diazo

reaction into 3,4,5-triodonitrobenzene. Condensation of the latter with the monomethyl ether of hydroquinone in methyl ethyl ketone solution in the presence of potassium carbonate gave the diphenyl ether (I) in which the nitro group was subsequently reduced and replaced by the cyano group by means of the Sandmeyer reaction. Reduction of the cyano group with anhydrous stannous chloride gave the aldehyde (II) which was condensed with hippuric acid in order to introduce the alanine side chain. The azlactone (III) which was first formed was hydrolysed and reduced and then demethylated and debenzoylated to give the diiodo acid (IV). The introduction of the additional iodine atoms was effected by the addition of a solution of iodine in potassium iodide to a solution of the ammonium salt. Liberation of the free acid on acidification gave D-thyroxine (V).

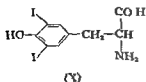


The resolution of the racemic compound presented considerable difficulties owing to its sparing solubility but in 1928 Harington⁷ was able to resolve DL 3,5 diiodothyronine (IV) into the two optically active forms into each of which the two additional iodine atoms were subsequently introduced.

An improved synthesis of thyroxine has recently been reported⁸ and an extension of this method to L tyrosine leads to L thyroxine without loss of optical activity.⁹ In this process L tyrosine is converted to 3,5 dinitro L tyrosine (VI) which is submitted to acetylation and esterification to give the ester (VII). Reaction with the monomethyl ether of hydroquinone in pyridine gives 3,5 dinitro 4-*p*-methoxyphenoxy N-acetyl L phenylalanine ethyl ester (VIII) which is then reduced to the diamine and submitted to the Sandmeyer procedure to give the diiodo ester (IX). Subsequent hydrolysis and iodination with iodine in ethylamine solution gives L thyroxine (V) ($[\alpha]_D -5.7$ in N ethyl alcoholic sodium hydroxide). The overall yield from L tyrosine is 26 per cent.



In addition to thyroxine the thyroid gland contains 3,5 diiodotyrosine (λ)¹⁰ an amino acid which had been previously isolated from coral. This amino acid is regarded as a precursor in the biogenesis of thyroxine. By the use of radioactive iodine as a tracer it has been shown that iodine administered as iodide becomes located in the thyroid gland being incorporated firstly in 3,5 diiodotyrosine and finally in the thyroxine component of the protein iodothyroglobulin. In 1939 Ludwig and von Mutzenbecher¹¹ obtained physiologically active compounds



by the iodination of casein and similar proteins and it has been shown that in these reactions 3,5 diiodotyrosine and thyroxine are formed^{12, 13}. The direct conversion of 3,5 diiodotyrosine into thyroxine was also established experimentally by von Mutzenbecher¹⁴ and by Harington¹⁵.

PARATHYROIDS

The hormone of the parathyroids is a protein which is decomposed by trypsin. It is therefore not active when given by mouth. Potent extracts of the parathyroids have been prepared independently by Collip¹⁶, by Twerdy¹⁷ and by Dyer¹⁸ by boiling the fresh glands for a limited period of time with dilute acid. Little is known of the chemistry of the active principle.

PANCREAS

The secretion of those portions of the pancreas termed the islets of Langerhans is the hormone insulin.

Insulin. Insulin can be isolated from the pancreas of the ox or pig and the islets derived from the pancreas of certain fish also provide a useful source. The hormone possesses the normal characteristics of a protein and it was thus difficult to obtain in crystalline form. Various processes have been used for the isolation of the hormone and the following method due to Scott and Parker¹⁹ is typical. The fresh minced pancreas from the ox is allowed to stand overnight in acidified aqueous alcohol. The extract is separated in a centrifuge and made alkaline with ammonia. The inactive precipitate is filtered off, the filtrate acidified with sulphuric acid and the alcohol removed under reduced pressure. The fats which separate are filtered off and the active principle is liberated from the filtrate by the addition of salt. The precipitated material is further purified by repeated carefully controlled precipitation from solution. The original literature should be consulted for further details of this and other processes of isolation and purification. Obtained in this manner insulin is an amorphous powder but Abel in 1926²⁰ and Harington and Scott in 1929²¹ reported the preparation of crystalline insulin by precipitation from a highly buffered solution in the presence of either large quantities of brucine acetate and pyridine or saponin. The crystals (see Fig. 24) were found to contain zinc^{22, 23} but the exact manner in which the zinc is combined is uncertain and contrary to earlier reports the percentage of zinc is not strictly constant^{24, 25}. Crystalline insulin turns brown at 216° and melts with decomposition at 233°. It is optically active and levorotatory. The iso electric point is at pH ≈ 3 but the best conditions for crystallisation are probably within the range pH 5.8 to 6.3. As an amphoteric substance insulin is soluble in both acids and alkalis but it is not stable in alkaline solution. Salts such as the hydrochloride, sulphate and picrate can be obtained as amorphous powders. It gives the normal reactions

for proteins such as the biuret Millon ninhydrin and xanthoproteic tests and contains about 15 per cent of nitrogen and about 3.3 per cent of sulphur. It is sensitive to proteolytic oxidation and reduction enzymes. The molecular weight of insulin has been given as 20 000 by chemical methods but the ultracentrifuge method gives values of 46 000 to 48 000 and the X ray method a value of 36 000. Viscosity measurements give values between 40 000 and 50 000. The unit cell

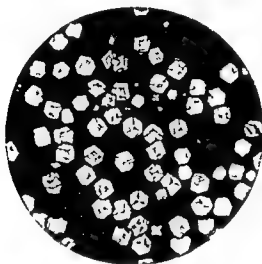


FIG. 1
INSULIN CRYSTALS ($\times 200$)

from X ray measurements was shown to possess trigonal symmetry which indicated a three unit structure with a molecular weight of 12 000 for each unit. Gutfreund²⁶ considers that the real molecular weight of insulin is 12 000 and that in neutral solution four such molecules are loosely bound together into a large molecule of molecular weight 48 000 which dissociates on dilution or at extreme pH values. In the crystalline form three such molecules are combined in the unit cell. On the basis of a molecular weight of 12 000 coupled with the results of the application of modern methods of amino acid analysis Sanger²⁷ has given the figures in Table V for the number of the various amino acid units in the insulin molecule.

This analysis accounts for about 98.5 per cent of the insulin molecule. The manner in which these units are arranged in the molecule is largely unknown although the presence of certain amino acid sequences has been demonstrated. All attempts to separate from insulin an active compound of comparatively low molecular weight have been unsuccessful^{28, 29, 30} and even mild hydrolysis results in loss of activity.

TABLE V
AMINO ACIDS IN THE INSULIN MOLECULE

Amino acid	Number of Units per Molecule
Glutamic Acid	15
Leucine	12
Cystine/2	12
Aspartic Acid	12
Tyrosine	8
Valine	8
Glycine	7
Alanine	7
Proline	6
Serine	6
Aspartic Acid	6
Histidine	4
Proline	3
isoLeucine	3
Arginine	2
Lysine	2
Threonine	2

While it has been shown that the presence of certain groups in the molecule is essential for activity and that the presence of others is not it remains true that the specific activity of insulin must be regarded as a property of the molecule as a whole or of some labile degradation product which has so far escaped detection.

Insulin cannot be administered by mouth owing to its destruction by proteolytic enzymes. Many successful attempts have been made to obtain derivatives which give rise to a more prolonged action after injection. Hagedorn²¹ showed that insulin can be combined with other substances to give products which have an isoelectric point nearer to that of tissue fluids than has insulin itself. In this way active compounds of lower solubility are obtained. Compounds or mixtures of insulin with saponins, bile acids and blood serum have been recommended. Among the more successful substances of this type may be mentioned protamine insulin which is a combination of insulin hydrochloride with protamine obtained from the sperm of rainbow trout. This substance is more slowly absorbed and a further improvement can be obtained by the addition of a small quantity of zinc chloride²² (see pages 23 and 177).

PITUITARY

The pituitary gland or hypophysis consists of two parts the posterior lobe and the anterior lobe which in nearly all animals are assembled together in one organ. In the whale the two parts are anatomically independent.

Posterior Lobe. At least two hormones are produced in the posterior lobe. Of these one causes the contraction of the uterus and

for proteins such as the biuret Millon ninhydrin and xanthoproteic tests and contains about 15 per cent of nitrogen and about 3.3 per cent of sulphur. It is sensitive to proteolytic oxidation and reduction enzymes. The molecular weight of insulin has been given as 20 000 by chemical methods but the ultracentrifuge method gives values of 46 000 to 48 000 and the X ray method a value of 36 000. Viscosity measurements give values between 40 000 and 50 000. The unit cell

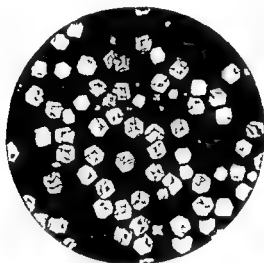


FIG. 24
INSULIN CRYSTALS ($\times 200$)

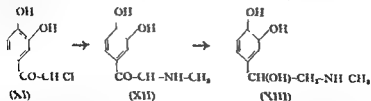
from X ray measurements was shown to possess trigonal symmetry which indicated a three unit structure with a molecular weight of 12 000 for each unit. Gutfreund²⁶ considers that the real molecular weight of insulin is 12 000 and that in neutral solution four such molecules are loosely bound together into a large molecule of molecular weight 48 000 which dissociates on dilution or at extreme pH values. In the crystalline form three such molecules are combined in the unit cell. On the basis of a molecular weight of 12 000 coupled with the results of the application of modern methods of amino acid analysis Sanger²⁷ has given the figures in Table V for the number of the various amino acid units in the insulin molecule.

This analysis accounts for about 98.5 per cent of the insulin molecule. The manner in which these units are arranged in the molecule is largely unknown although the presence of certain amino acid sequences has been demonstrated. All attempts to separate from insulin an active compound of comparatively low molecular weight have been unsuccessful^{28, 29, 30} and even mild hydrolysis results in loss of activity.

the alcoholic filtrate is concentrated. Addition of concentrated ammonia to the residual aqueous solution liberates free adrenaline which may be purified by solution in an alcoholic solution of oxalic acid filtration dilution with water and addition of ammonia. Owing to the susceptibility of adrenaline to oxidation it is essential to carry out the extraction processes under reduced pressure or in an atmosphere of carbon dioxide or under a layer of light petroleum. Owing to the simplicity of the process the natural product continues to be used in considerable quantities.

Adrenaline ($C_9H_{13}O_3N$) Adrenaline (XIII) is a colourless crystalline compound m.p. 211° which is almost insoluble in water and most organic solvents but dissolves readily in acids and alkalis. It is thus an amphoteric substance which displays both basic and acidic properties. It is comparatively stable in acid solution but in the presence of alkalis it is readily oxidised. It gives a green colour with aqueous ferric chloride solution and possesses strong reducing properties. The natural hormone is levorotatory ($[\alpha]_D -53$ in aqueous hydrochloric acid). The racemic form is less active physiologically since (+) adrenaline has only one twelfth to one sixteenth the activity of the (−) isomeride.

Adrenaline (XIII) is a derivative of catechol containing a secondary alcohol group and also a methylamino group. Evidence for this constitution was obtained by degradation experiments and the constitution was confirmed by synthesis which was achieved almost simultaneously by Dakin⁴¹ and by Stolz⁴². 3,4-Dihydroxy- ω -chloroacetophenone (XI) was prepared by the action of chloroacetic acid on catechol in the presence of phosphorus oxychloride a reaction which was subsequently investigated and improved by Ott⁴³. By the action of an excess of a concentrated aqueous solution of methylamine on the chloro-ketone suspended in alcohol 3,4-dihydroxy- ω -methylaminoacetophenone (XII) was obtained and this on reduction gave adrenaline (XIII). Reduction can be effected either electrolytically by means of aluminium amalgam or catalytically with hydrogen and palladium. The



resulting (±) adrenaline can be resolved by means of its bitartrate. (+) adrenaline (+) tartrate being much more soluble in methyl alcohol than (−) adrenaline (+) tartrate⁴⁴. Further purification of (−) adrenaline (+) tartrate is effected by crystallisation from 95 per cent methyl alcohol. The crude (+) adrenaline (+) tartrate is

the other constriction of the capillaries and contraction of the intestines. Both extracts have an antidiuretic effect and are used in the treatment of diabetes insipidus. These hormones are proteins.

Anterior Lobe The anterior lobe of the pituitary is a gland of the greatest importance which has far reaching influences on the functions of many other glands. Extracts of the anterior lobe appear to contain a number of distinct active principles some of which involve the metabolism of the whole body resulting in extreme cases either in dwarfism or gigantism. More specific effects are also produced on other glands such as the pancreas, thyroid, parathyroids and adrenals and the extracts contain three gonadotrophic substances. Little is known concerning the chemistry of these substances all of which appear to be proteins.

Of the various hormones associated with the anterior lobe most attention has been given to the gonadotrophic factors. A product with high luteinising activity can be obtained from human pregnancy urine by precipitation with phosphomolybdic acid. The precipitate is subsequently dissolved in ammonium baryta is added. After filtration the hormone is precipitated by the addition of alcohol and ether.³⁴ Various other methods for the preparation of the hormone have also been reported.³⁵⁻³⁸ An alternative source of the follicle stimulating hormone is the serum of pregnant mares from which a highly active preparation can be obtained by removal of the inactive proteins with salicylsulphonic acid, followed by dialysis and fractional precipitation with acetone.³⁷

ADRENALS

The adrenal glands consist of two parts the medulla and the cortex. The medulla is the source of the hormones adrenaline and noradrenaline while the cortex provides a large number of steroid hormones (see page 77).

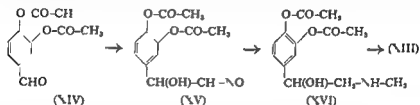
Adrenal Medulla In 1894 Oliver and Schafer³⁶ found that when an extract of the adrenal glands was injected intravenously into animals a marked rise in blood pressure resulted and in 1901 the active principle responsible for this action was isolated in crystalline form independently by Takamine³⁹ and by Aldrich.⁴⁰ This active principle which was shown to be derived from the medulla and was given the name adrenaline was the first hormone to be isolated in a pure form.

The hormone can be readily isolated in almost quantitative yield from the adrenal glands of oxen by utilising its basic properties. The minced glands are extracted with hot dilute acid and after further heating the extract to coagulate most of the proteins it is filtered and concentrated under reduced pressure. The concentrated residue is treated with two or three times its volume of alcohol and after filtration

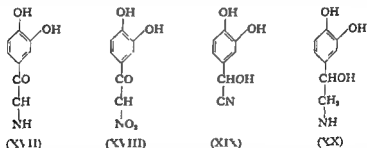
REFERENCES

- 1 KENDALL E C *J biol Chem* 1919 39 125
- 2 KENDALL E C and OSTERBURG A E *J biol Chem* 1919 40 265
- 3 HARRINGTON C R *Biochem J* 19 11 20 293
- 4 HARRINGTON C R *Biochem J* 1930 24 456
- 5 HARRINGTON C R *Biochem J* 1926 20 300
- 6 HARRINGTON C R and BARGER G *Biochem J* 1927 21 169
- 7 HARRINGTON C R *Biochem J* 1928 22 1429
- 8 BORROWS E T CLAYTON J C and HEMS B A *J chem Soc* 1949 S199
- 9 CHALMERS J R DICKSON G T ELKS J and HEMS B A *J chem Soc* 1949 3424
- 10 HARRINGTON C R and RANDALL S S *Biochem J* 1929 23 373
- 11 LUDWIG W and von MUTZENBECHER P *Z physiol Chem* 1939 258 195
- 12 HARRINGTON C R and RIVERS R P *Nature Lond* 1939 144 205
- 13 REINEKE F P and TURNER C W *J biol Chem* 1943 149 555
- 14 von MUTZENBECHER P *Z physiol Chem* 1939 261 253
- 15 HARRINGTON C R *J chem Soc* 1944 193
- 16 COLLIP J B *J biol Chem* 1925 63 305
- 17 TWEEDY W M *J biol Chem* 1930 88 649
- 18 DYER F J *J Physiol* 1936 86 3P
- 19 SCOTT D A and PARKER H *Proc roy Soc Canada* 1932 26 311
- 20 ABEL J J *Proc nat Acad Sci* 1926 12 132
- 21 HARRINGTON C R and SCOTT D A *Biochem J* 1929 23 384
- 22 SCOTT D A *Biochem J* 1934 28 1592
- 23 ROMANS D G SCOTT D A and FISHER A M *Industr Engng Chem* 1940 32 908
- 24 COHN E J FERRY J D LIVINGOOD J J and BLANCHARD M H *J Amer chem Soc* 1941 63 17
- 25 SAIYUN M *J biol Chem* 1941 138 487
- 26 GUTTFREUND H *Biochem J* 1948 42 544
- 27 SANGER F *Rep Prog Chem* 1948 45 283
- 28 HARRINGTON C R and NEUBERGER A *Biochem J* 1936 30 809
- 29 HARRINGTON C R and MEAD T M *Biochem J* 1936 30 1598
- 30 FREUDENBERG K *et al Z physiol Chem* 1931 202 128
- 31 HAGEDORN H C *J Amer med Ass* 1936 106 177
- 32 SCOTT D A and FISHER A M *J biol Chem* 1936 114 100XVIII
- 33 SCOTT D A and FISHER A M *J Pharmacol* 1936 58 78
- 34 SCHWEISLER H Deutsches Reichs Patent 588047
- 35 St LOUIS UNIVERSITY Missouri U S A British Patent 406531
- 36 GURIN S BACHMAN C and WILSON D W *J Biol Chem* 1939 128 525
- 37 RINDERKNECHT H NOBLE R L and WILLIAMS P C *Biochem J* 1939 33 381
- 38 OLIVER G and SCHAFER B A *J Physiol* 1894 16 1 1895 III 230
- 39 TAKAMINE J *Amer J Pharm* 1901 5 523
- 40 ALDRICH T B *Amer J Physiol* 1901 5 457
- 41 DAKIN H D *Proc roy Soc Series B* 1905 76 491
- 42 STOLZ F *Ber dtsh chem Ges* 1904 37 414J
- 43 OTT E *Ber dtsh chem Ges* 1926 59 1068
- 44 FLACHER F *Z physiol Chem* 1908 58 189
- 45 NAGAI N British Patent 118,98
- 46 HOLTON P *Nature Lond* 1949 163 217
- 47 GOLDENBERG M FARMER M ALSTON H J and CHARGAFF E C *Science* 1949 109 534
- 48 MEISTER LUCIUS and BRUNING Deutsches Reichs Patent 157300 193634 195814
- 49 TAINTER M L TULLAR B F and LUDUENA F P *Science* 1948 107 39

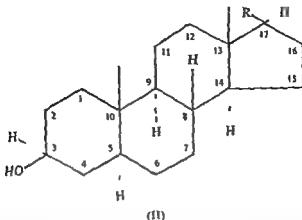
racemised by heating with hydrochloric acid and further resolution as before gives more (—) adrenaline. This process may be repeated until practically the whole of the synthetic product has been converted into the physiologically more active *lævo* form identical with the compound isolated from the medulla. In an alternative synthesis due to Nagai⁴⁵ nitromethane is condensed with the diacetyl derivative of proto catechuic aldehyde (XIV) and the reduction of the product (XV) with zinc dust and acetic acid in the presence of formaldehyde gives the diacetyl derivative of adrenaline (XVI) which may then be subjected to acid hydrolysis to give adrenaline (XIII).



Noradrenaline ($\text{C}_8\text{H}_{11}\text{O}_3\text{N}$) The presence in the medulla of the primary amine noradrenaline (XX) had long been suspected but it was not until 1949 that Holton⁴⁶ reported its presence in adrenal medullary tumours and its presence in the normal medulla was established shortly afterwards⁴⁷. It occurs as the (—) form. Racemic noradrenaline had been synthesised many years earlier by a variety of methods such as the reduction of either ω amino 3,4 dihydroxyacetophenone (XVII) ω nitro 3,4 dihydroxyacetophenone (XVIII) or the cyanhydrin of protocatechuic aldehyde (XIX)⁴⁸ and it had been introduced



under the name arterenol as a substitute for adrenaline but with little success. Its resolution has recently been reported⁴⁹. Racemic noradrenaline (XX) is a crystalline solid m.p. 191° with decomposition which is sparingly soluble in water, ethyl alcohol and ether. It is soluble in dilute acids and alkalis and yields a water soluble hydrochloride (m.p. 141°) and oxalate (m.p. 175°). (—) Noradrenaline melts at 216.5° to 218° corr. ($[\alpha]_D^{25} -37.3$) and its hydrochloride at 146° to 147° ($[\alpha]_D^{25} -39$).



at positions 3 5 8 9 10 13 14 and 17. Such a structure will be capable of existence in 2^8 or 256 stereoisomeric forms. Fortunately the main variations in stereochemical configuration encountered in natural products of the types under consideration are confined to the carbon atoms at positions 3 and 5 and the relative configurations of rings A and B. The configurations between rings B and C and between rings C and D appear to be the same in nearly all steroids with the exception of the cardiac aglycones and therefore do not call for detailed treatment here.

The conventions usually adopted for the description and representation of substituted steroids have been expressed clearly by Shoppee¹. The configuration of substituents at the nuclear carbon atoms is represented by the suffix (α) or (β) according to whether the substituent lies below the plane of the paper or projects forward from the plane of the paper (as for example with the 3 hydroxyl group in cholesterol). Further whenever it is necessary to indicate a specific configuration (β) orientation is represented in formulae by a full line bond whereas (α) orientation is represented by a broken line bond. Thus in formula (II) the hydroxyl group at position 3 the methyl group at positions 10 and 13 the group R at position 17 and the hydrogen atom at position 8 are all (β) orientated and extend above the general plane of the ring system whereas the hydrogen atoms at positions 3 5 9 14 and 17 are all (α) orientated and lie below the plane of the ring system. An excellent report of our knowledge of the stereochemical configuration of the steroid nucleus and of nuclear substituents has been published by Shoppee¹.

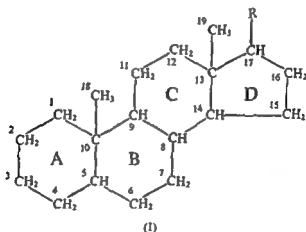
STEROLS

The sterols are constituents of animal and plant fats and oils. They are neutral stable crystalline compounds which occur partly in the free state and partly esterified with higher fatty acids. The usual method

CHAPTER IV

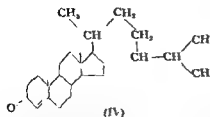
CHEMISTRY OF THE STEROID HORMONES

THE steroids are a group of compounds widely distributed in nature the structures of which are based on the reduced *cyclopentenophenanthrene* system. The parent system consists of four fused ring structures assembled as illustrated in formula (I) in which the group R is a saturated or unsaturated hydrocarbon radical. The group R is referred to as the side chain, the remainder of the molecule being termed the *nucleus*. The four ring systems which are usually though not always fully reduced are designated A, B, C and D and the carbon atoms are numbered as indicated. The sex hormones and the hormones of the



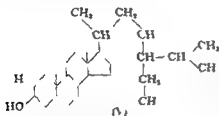
adrenal cortex come within this group and among other types of compounds which are included are the sterols, the bile acids, the aglycones of the cardiac glycosides and the steroid sapogenins. These various groups of compounds are thus closely related to the hormones and many of them are important as starting materials for the commercial preparation of the hormones. They are also of value in the elucidation of the stereochemical configurations of the hormones.

The stereochemistry of the steroids is a subject of considerable complexity which renders the total synthesis of any single member of this class a matter of great practical difficulty. If for purposes of illustration the fully saturated structure (II) is considered it will be observed that the molecule contains eight asymmetric carbon atoms, namely those



in ring A. This is a characteristic transformation associated with the oxidation of all 3 hydroxy steroids which contain a double bond between positions 5 and 6.

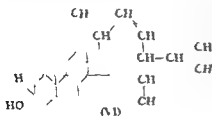
known in three forms termed α , β and γ sitosterol. The main constituent β sitosterol contains a secondary alcohol group at position 3 and an ethenoid link between positions 5 and 6. On oxidation it



Sitosterol ($C_{29}H_{50}O$) (V) the main sterol of plants is known in three forms termed α , β and γ sitosterol. The main constituent β sitosterol contains a secondary alcohol group at position 3 and an ethenoid link between positions 5 and 6. On oxidation it gives β sitostenone which has a ketonic group at position 3 and in which the double bond now occupies the position between the carbon atoms at positions 4 and 5. It differs from cholesterol in the side chain attached to position 17 which now consists of a satu-

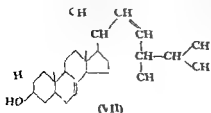
rated isodecyl group $C_{10}H_{21}$. Hydrogenation of β sitosterol gives stigmasterol (sitostanol) $C_{29}H_{50}O$.

Stigmasterol ($C_{29}H_{50}O$) (VI) is found with β sitosterol in soya



and calabar beans. It differs from β sitosterol only in having a double bond in the hydrocarbon side chain and on hydrogenation it takes up two molecular proportions of hydrogen to give stigmasterol identical with that obtained by the hydrogenation of β sitosterol.

Ergosterol ($C_{28}H_{44}O$) (VII) is the main steroid constituent of yeast. It mainly differs from cholesterol in having three double bonds instead of one. One of these additional double bonds is situated between



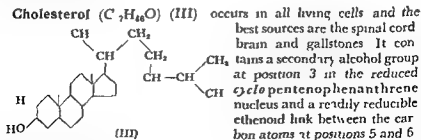
the carbon atoms at positions 7 and 8 and the other resides in the hydrocarbon side chain attached to the carbon atom at position 17 which contains nine carbon atoms. Three molecular proportions of hydrogen are absorbed on catalytic

of isolation consists in subjecting a neutral extract to hydrolysis with alcoholic sodium or potassium hydroxide and extracting the unsaponifiable matter with ether or light petroleum. In many cases the sterols isolated from natural sources consist of mixtures of closely related compounds. All sterols contain a (β) orientated hydroxyl group attached to position 3 in the reduced cyclopentenophenanthrene system. Sterols obtained from animal sources are termed zoosterols and those from plants phytosterols. The more important sterols are listed in Table VI.

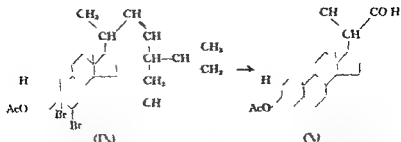
TABLE VI
STEROLS

Name	Formula	Number of Double Bonds	Mp	$[\alpha]_D$	Occurrence
Cholesterol	$C_{27}H_{48}O$	1	150	-38.8	Animal cells
Ergosterol	$C_{28}H_{44}O$	3	163	-133	Yeast Ergot
β -Sitosterol	$C_{27}H_{48}O$	1	146	-42.4	Plants
Stigmasterol	$C_{27}H_{46}O$	2	170	-45	Soya bean
Zymosterol	$C_{27}H_{46}O$	2	110	+47.3	Yeast
Fucosterol	$C_{29}H_{50}O$	2	124	-38.4	Algae
Coprosterol (Coprostanol)	$C_{27}H_{48}O$	0	102	+23.5	Feces

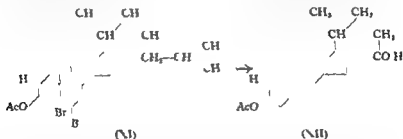
In these compounds the group R of formulae (I) and (II) consists of a hydrocarbon chain containing from eight to ten carbon atoms and where is coprosterol (coprostanol) is fully saturated the other members contain from one to three double bonds. Cholesterol, β -sitosterol and stigmasterol have been used as starting materials for the synthesis of sex hormones.



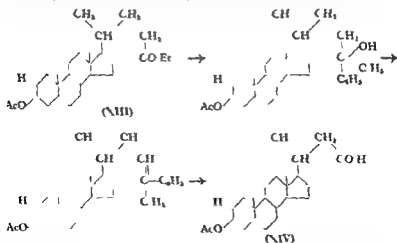
The side chain attached to the carbon atom at position 17 is a saturated iso-octyl group C_8H_{17} . Oxidation of cholesterol gives the 3-keto steroid known as cholestenone (IV) in which the double bond has moved from between positions 5 and 6 in ring B to between positions 4 and 5.



Further in the oxidation of cholesterol acetate dibromide (VI) by means of chromic acid Δ^5 3(β) acetoxy Δ^5 cholenic acid (VIII) is formed as a by product and this acid can be degraded to 3(β) acetoxy Δ^5 bisnorcholenic acid (IX) by means of the Wieland Barbier process



In this method an ester (XIII) of 3(β) acetoxy Δ^5 -cholenic acid is treated with a Grignard reagent such as phenylmagnesium bromide and the resulting tertiary alcohol is dehydrated and oxidised to give 3(β) acetoxy Δ^5 norcholenic acid (XIV) as indicated below

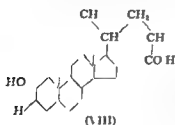


This sequence of reactions is then repeated on the ethyl ester of (XIV) which leads in turn to the production of 3(β) acetoxy Δ^5 bisnorcholenic acid

hydrogenation giving ergosterol and oxidation of ergostanyl acetate gives 3(β) acetoxy nor 5 allocholic acid (see below)

BILE ACIDS

The bile acids are normal constituents of the secretions of the liver of man and animals. Like the sterols the bile acids contain a hydroxyl group attached to the carbon atom at position 3 but this is now (α) orientated and in place of the hydrocarbon chain attached to the carbon atom at position 17 a shortened chain at this position now contains a carboxyl group which is responsible for the acidic properties of the molecule. The basic substance of this class may be regarded



as lithocholic acid or 3(α) hydroxy cholic acid (VIII). The main members of this series are given in Table VII and each can be converted to the parent substance cholic acid by oxidation of the hydroxyl group or groups to ketone groups followed by reduction by the Clemmensen method with amalgamated zinc and hydrochloric acid.

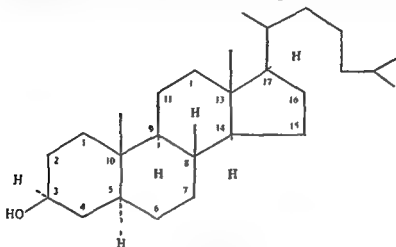
TABLE VII
BILE ACIDS

Name	Number of Hydroxyl Groups	Position of Hydroxyl Groups	Occurrence
Lithocholic Acid	1	3	Cattle Bile
Cholic Acid	3	3 7 12	Human Bile
Deoxycholic Acid	2	3 12	Human Bile
Hyodeoxycholic Acid	2	3 6	Pig Bile

A number of unsaturated steroid acids are encountered in the processes used for the preparation of sex hormones from sterols. Stigmasterol (VI) for example provides a suitable starting material for the preparation of a steroid acid since it contains a vulnerable double bond in the side chain which will be susceptible to oxidation. Thus if the hydroxyl group in stigmasterol is protected by acetylation and the nuclear double bond by bromination as in stigmasteryl acetate dibromide (IX) subsequent ozonolysis and debromination of the product gives 3(β) acetoxy Δ^5 bisnorcholic acid (X).

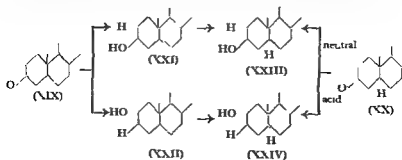
STEREOCHEMISTRY

Brief reference has already been made to the complexity of the stereochemistry of the sterols and related compounds. In the molecule of cholestanol (XVIII) the rings A and B and B and C and C and D are joined together in the *trans* configuration and the methyl groups or hydrogen atoms attached to the carbon atoms at positions 5 and 10, 8 and 9 and 13 and 14 are also in the *trans* configuration.



(XVIII)

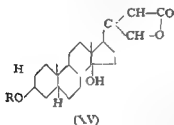
When Δ^5 cholestenone (XIX) is reduced to the corresponding alcohol by means of hydrogen in presence of Raney nickel catalyst the product is a mixture of cholesterol (XXI) and *epi*cholesterol (XXII) a new asymmetric centre having been introduced at the carbon atom at position 3. Both *sterols* can be further reduced at the double bond by means of hydrogen in the presence of a platinum catalyst to give cholestanol (XXIII) and *epi*cholestanol (XXIV) which can also be obtained directly from cholestanone (XX) by reduction under neutral and acid conditions respectively. These changes may be represented as follows:



acid (X) identical with the acid obtained from stigmasteryl acetate dibromide by ozonolysis

AGLYCONES OF THE CARDIAC GLYCOSIDES

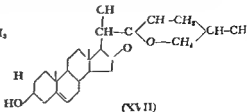
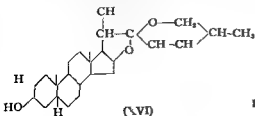
The digitalis glycosides occur in the leaves of varieties of digitalis (*Digitalis purpurea* *D. lanata*) and closely related compounds are also found in other plants e.g. strophanthus. They have a powerful action on the heart. The glycosides isolated from *D. purpurea* and *D. lanata* are known as digitoxin (XV R=digitoxose residue), gitoxin and digoxin and can be hydrolysed to the aglycones digitoxigenin ($C_{27}H_{44}O_4$) (XV R=H), gitoxigenin ($C_{27}H_{44}O_4$) and digoxigenin ($C_{27}H_{44}O_5$) respectively together with the sugar digitoxose. The sugar free



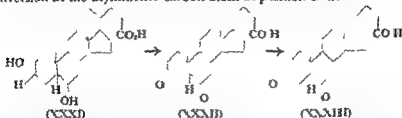
compounds digitoxigenin, gitoxigenin and digoxigenin are known to be lactones with a steroid nucleus largely as a result of the pioneer investigations of Jacobs and of Windaus and Stoll. In digitoxigenin, gitoxigenin and digoxigenin a secondary alcohol group occurs at position 3 in the reduced cyclopentenophenanthrene system and the most characteristic feature of these compounds is the occurrence of an unsaturated lactone ring attached to the carbon atom at position 17 upon which the physiological activity depends.

SAPONGENINS

The saponins are a group of glycosides which occur widely in plants and the aglycones termed sapogenins derived from them belong to two groups. Some give 3-methyl-1,2-cyclopentenophenanthrene (Diel hydrocarbon) on dehydrogenation and are therefore closely related to the steroids, whereas others give 1,2,7-trimethylnaphthalene (sapotalene) on dehydrogenation with selenium, which indicates a close relationship to the triterpenes. To the former class belong tigogenin ($C_{27}H_{44}O_3$), gitogenin ($C_{27}H_{44}O_4$) and digitogenin ($C_{27}H_{44}O_5$). A similar compound sarsasapogenin ($C_{27}H_{44}O_3$) (XVI) obtained from sarsaparilla (*Smilax* species) has been used for the synthesis of sex hormones. Diosgenin ($C_{27}H_{42}O_3$) (XVII) isolated from the roots of *Trillium erectum* and from *Dioscorea tororo* (Makino) has also been used for similar purposes.



The significance of the stereochemical possibilities encountered in the steroids is perhaps best illustrated by reference to cholesterol and the bile acids which have been shown to belong to two different stereochemical series. For example, hyodeoxycholic acid from hog bile is a 3,6-dihydroxycholanic acid (XXXI) of the 5 *normal* series, as also is the diketone acid (XXXII) obtained on oxidation, but the latter compound is capable of rearrangement by a keto-enol change in the presence of acids or bases to a more stable stereoisomere (XXXIII) of the 5 *allo* series, which change must obviously be attributed to an inversion at the asymmetric carbon atom at position 5, thus:



It was thus established that in the cholanic acids the rings A and B are in the *cis* configuration, thus belonging to the *normal* or coprostanic series, whereas in the 5 *allo* acids the configuration is of the *trans* type encountered in derivatives of the cholestane series. As previously stated, the bile acids also differ from the sterols in having an (α /orientated hydroxyl) group at position 3. To the former series belong also the hydrocarbons α -etiocholan and pregnane, while androstane and 5 *allo* pregnane belong to the latter.

There is abundant evidence that oestrogenic and androgenic progestational and cortical activity is highly sensitive to stereochemical structural changes. Progesterone and deoxycorticosterone, for example, lose their activity in the corresponding 17 *iso*-compounds in which only the stereochemical configuration at the carbon atom at position 17 is changed. In a similar manner *cis*-testosterone and β -oestradiol possess only a fraction of the activity associated with testosterone and α -oestradiol respectively.

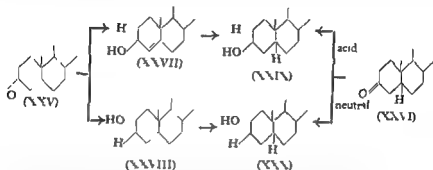
SEX HORMONES

The sex hormones are the natural secretions of the gonads or glands which are responsible for the sexual processes and for the secondary characteristic features which differentiate the male and female of a species. These compounds are sometimes referred to as the secondary sex hormones, since they are controlled by the protein-like substances secreted into the blood from the anterior lobe of the pituitary, which are sometimes called the primary sex hormones.

The sex hormones are of three types:

- (a) Estrogens (female or follicular hormones)
- (b) Androgens (male hormones)
- (c) Progesterone (the hormone of the corpus luteum)

In a similar manner Δ^4 cholestenone (XXV) may be reduced with aluminium isopropoxide to give a mixture of 3(β) hydroxy Δ^4 cholestene (allocholesterol) (XXVII) and 3(α) hydroxy Δ^4 cholestene (epiallocholesterol) (XXVIII) which on further catalytic reduction give coprosterol (coprostanol) (XXIX) and epicoprosterol (epicoprostanol) (XXX) respectively. The latter are also obtained by reduction of coprostanone (XXVI) in acid and neutral media thus



Precipitation of a digitonide on the addition of a solution of digitonin in alcohol (90 per cent) provides a rough diagnostic test for 3(β) hydroxysteroids such as cholesterol, cholestanol and coprosterol (coprostanol) in which the hydroxyl group is on the same side of the ring as the methyl group at position 10. Epicholestanol and epicoprosterol (epicoprostanol) do not give such precipitates. The configuration taken up by the 3 hydroxyl group in the reduction of cholestanone and coprostanone follows the Auwers Skita Rule which was deduced from a study of the reduction of the decalones and which states that under neutral conditions *trans* compounds are usually formed whereas reduction under acid conditions gives *cis* compounds. Thus in cholestanol the hydroxyl group at position 3 and the hydrogen at position 5 are in the *trans* configuration since the cholestanol was obtained from cholestanone by catalytic reduction in neutral solution. The above considerations show that the isomerides cholestanol (or dihydrocholesterol) and coprosterol (coprostanol) differ only in the stereochemical configuration at position 5 since the same isomeric relationship is maintained in the saturated hydrocarbons cholestane and coprostan. In cholestane the configuration of rings A and B is of the *trans* decalin type whereas in coprostan the configuration is of the *cis* decalin type. In cholestane the hydrogen atom at position 5 is in the *trans* position with reference to the methyl group at position 10 and this configuration gives rise to the 5 *allo* series. In coprostan the hydrogen atom at position 5 and the methyl group at position 10 are in the *cis* configuration which gives rise to the 5 *normal* series. The hydroxyl group at position 3 in cholesterol and in cholestanol is thus regarded as being in the *cis* position with reference to the methyl group at position 10.

Little is known concerning the synthesis of the steroid hormones in the body. It is known that plant sterols derived from the diet do not provide a source for the cholesterol found in animals but are excreted in the faeces. Experiments with deuterium in place of hydrogen in water and in acetic acid fed to rats and mice have shown that cholesterol can be synthesised in the body from such simple molecules as those of water and acetic acid². In similar experiments using deuterium as a tracer it has been shown that cholesterol can be converted into cholic acid in the dog³ and into progesterone in the human female⁴.

Oestrogens

Isolation Several methods have been described for the isolation of the oestrogenic hormones from pregnancy urine. In one method the urine is extracted with ether and the ethereal extract is evaporated. The residue is dissolved in methyl alcohol and the solution is shaken with light petroleum. The alcoholic solution is then diluted and extracted with ether and the extract evaporated. The residue is treated with a mixture of alcohol (60 per cent) and benzene and whereas the alcoholic layer contains most of the oestriol the benzene layer retains the oestrone. The crude hormones are hydrolysed with hydrochloric acid and taken up in ether. The ethereal solution is washed with aqueous sodium carbonate solution and extracted with aqueous sodium hydroxide solution which removes the phenolic hormones and leaves the inactive pregnanediol. The hormones are precipitated from alkaline solution by the addition of acid and taken up in ether. The oestrone may be separated by high vacuum distillation whereas the oestriol can be purified by precipitation from alcoholic solution by the addition of ether⁵.

Improved methods of isolation have been reported by *Marrion*⁶ in which the urine is first concentrated and then extracted at pH 2.5 to 3.0 with *n*-butyl alcohol after saturation with salt. The hormones are extracted as before with aqueous alkali and then purified. The oestriol is thus obtained as the sodium salt of the glucuronide. *Girard* and *Sandulesco*⁷ utilised the Girard Reagent T (trimethylaminoacetylhydrazide hydrochloride) for the isolation of oestrone with which it forms a water soluble derivative. Other workers have used the semi-carbazone and the addition compound with quinoline for the isolation and purification of oestrone.

In isolating oestrone from the urine of pregnant mares it is necessary to make the urine strongly acid and the subsequent procedure varies from that recommended for human pregnancy urine. *Dossy* and his collaborators⁸ employed a method in which the hormone is precipitated with benzoic acid. *Beall* and *Marrion*⁹ used toluene for the initial extraction and after various partitions between organic solvents and alkali the oestrone was precipitated as a mercury complex. The original literature should be consulted for details of these processes. A method

The oestrogens may be regarded as derivatives of oestrane (XXXIV) which contains eighteen carbon atoms and in which ring A contains three double bonds and is therefore aromatic in type. In a similar manner the androgens are based on the hydrocarbon androstane (XXXV) which contains nineteen carbon atoms and is fully saturated while progesterone is based on 5 *allo* pregnane (17[β] ethyl androstane) (XXXVI), a fully saturated hydrocarbon containing twenty one carbon atoms. The sex hormones and certain of their excretion products are given in Table VIII.

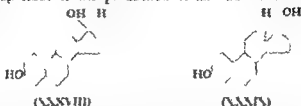


TABLE VIII
SEX HORMONES AND EXCRETION PRODUCTS

Name	Formula	Mp corr	$[\alpha]_D$	Occurrence
Oestrogens				
(+) Oestrone	$C_{18}H_{22}O_2$	259	+170 in dioxan	{ Human pregnancy urine urine of pregnant mares bull's urine stallion's urine adrenal cortex
α Estradiol	$C_{18}H_{24}O$	176 to 178	+81 in alcohol	{ Hog ovaries urine of pregnant mares
Estriol	$C_{21}H_{26}O_3$	281	+61 in alcohol	{ Human pregnancy urine
(+) Equilenin	$C_{18}H_{22}O_2$	238 to 259	+87 in dioxan	{ Urine of pregnant mares
Equilenin	$C_{18}H_{22}O_2$	238 to 240	+308 in dioxan	{ Urine of pregnant mares
Androgens				
Androsterone	$C_{19}H_{28}O_2$	184 to 185	+94.6 in alcohol	Male urine
Dehydroandrosterone	$C_{19}H_{26}O_2$	140 to 141 152 to 153	+10.9 in alcohol	Male urine
Testosterone	$C_{19}H_{28}O_2$	155	+109 in alcohol	Testes
Progesterone	$C_{21}H_{32}O$	129-121	+192	{ Sow ovaries placenta pregnancy urine adrenal cortex

A total synthesis of oestrone has been reported by Anner and Miescher (see page 81)

α -Oestradiol ($C_{18}H_{26}O_2$) 3:17 Dihydroxy $\Delta^{1,3,5}$ -oestratriene The reduction of oestrone to the secondary alcohol oestradiol was first reported by Schwenk and Hildebrandt in 1933¹⁹. Their product was probably a mixture of the two isomerides now known as α (XXXVIII) and β -oestradiol (XXXIX) since reduction of the carbonyl group leads to the production of an additional asymmetric



carbon atom at position 17*. The reduction product of oestrone was shown to be more potent in the Allen Doisy test than oestrone itself. Of the two isomerides α -oestradiol was found to be about five times more active than (+)-oestrone whereas β -oestradiol is stated to be much less active. Although α -oestradiol was first prepared synthetically by reduction of oestrone it was later isolated from the ovaries of sows by Doisy^{20, 21} who believed it to be the active oestrogenic principle of the follicular fluid. He regarded oestrone as a secondary product of less importance to the organism. The two isomeric oestradiols each contain two hydroxyl groups, one of which is phenolic in type and they give rise to a series of mono- and di-esters such as the 3-acetate (m.p. 136.5 to 137.5), 17-acetate (m.p. 215 to 217.5) and 3:17 diacetate (m.p. 127). Many of these esters possess a more persistent physiological action than free oestradiol and for this reason the monobenzoate which contains the benzoyleloxy group attached to the aromatic ring and the dipropionate were introduced into clinical practice. A method of preparing α -oestradiol from cholesterol has been reported by Inhoffen (see page 80).

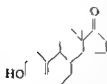
Oestriol ($C_{18}H_{24}O_3$) 3:16:17 Trihydroxy $\Delta^{1,3,5}$ -oestratriene Shortly after the isolation of oestrone from human pregnancy urine by Doisy and by Butenandt the isolation from the same source of a second oestrogenic substance with a higher melting point was reported by Marrian²². This substance (XL) was at first regarded as a hydrate of the hydroxy ketone oestrone and this hypothesis was confirmed when Butenandt²³ showed that when the new substance was distilled in a high vacuum in the presence of potassium hydrogen sulphate oestrone was formed with elimination of water, a conclusion which was later

* Compound (XXXVIII) called α -oestradiol by convention despite the fact that the hydroxyl group at position 17 has the (β)-orientation.

for the isolation of œstrone from the urine of stallions has been described by Cartland and his collaborators¹⁰

Whereas œstrone can be extracted completely with ether from a weakly alkaline solution much of the more acidic œstriol is not so extracted. This difference in acidic strength permits a nearly quantitative separation¹¹. The hormones equilin and equilenin were isolated from the urine of pregnant mares largely by the use of the Girard Reagent T followed by distribution methods and crystallisations.

œstrone ($C_{18}H_{26}O_2$) (+) œstrone 3-Hydroxy 17-keto- $\Delta^{1,2,3}$ œstratriene Ketohydroxyœstrin œstrone (XXXVII) is a phenolic



(XXXVII)

ketone. It was isolated in a pure crystalline form in 1929 by Doisy, Veler and Thayer¹ and independently by Butenandt¹². Early in the following year a similar substance was isolated by Dingemans, de Jongh, Kober and Laqueur¹³. In each instance the hormone was isolated from human pregnancy urine. œstrone has the normal chemical properties associated

with a phenolic ketone and various derivatives have been prepared such as the acetate (m.p. 126°), benzoate (m.p. 218° to 219°), methyl ether (m.p. 168.5° to 169°), oxime (m.p. 241° to 242°) and semicarbazone (m.p. 266° to 267°). As a phenol it couples with diazonium salts in alkaline solution. In 1930 Zondek¹⁴ showed that the urine of pregnant mares provided a richer source of œstrone than human pregnancy urine and this discovery led not only to the production of œstrone on a commercial basis with much improved methods of extraction and purification but also to the isolation of a number of closely related compounds such as equilin and (+) equilenin. At a later date Zondek¹⁵ showed that œstrone was also present in comparatively large quantities in the urine of stallions. The œstrogenic hormone content of urine from various sources is shown in Table IX. œstrone has also been isolated from the urine of normal men¹⁷ and the occurrence of œstrone in palm kernel oil has been reported by Butenandt and Jacobi¹⁸.

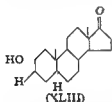
TABLE IX.
œSTROGEN CONTENT OF URINE

Source of Urine	œstrogen Content (mg. per l.)
Pregnant woman	2.1
Mature woman	0.042
Pregnant mare	10
Mature mare	0.070
Stallion	17
Bull	0.033

which acidic and phenolic compounds were removed by shaking with aqueous alkali. Steam distillation of the neutral chloroform extract removed volatile impurities and after boiling successively with alkali and acid the unsaponifiable material containing the hormone was purified by partition between solvents firstly between benzene and light petroleum and then between light petroleum and alcohol (60 per cent). The crude androsterone was purified through its oxime²⁷. Subsequently improvements in this procedure were introduced involving the use of the Girard Reagent T and chromatography²⁸.

The isolation of dehydrosoandrosterone from male urine has been described by Butenandt²⁹. Testosterone was isolated from testis tissue by David Dingemans, Freud and Laqueur³⁰ about 10 milligrams being obtained from 100 kilograms of tissue. The original literature should be consulted for further details of these processes.

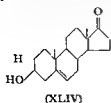
Androsterone ($C_{19}H_{30}O_2$) 3(α) Hydroxy 17 keto-androstane. Using the comb growth test as a guide Butenandt and Tscherning²⁷ were successful in isolating a pure crystalline male or androgenic hormone from human male urine. This compound to which the name androsterone (XLIII) was given proved to be fully saturated and



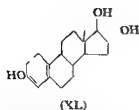
possessed a secondary alcohol group as well as a ketone group. The analytical figures indicated that it is a steroid ketone bearing a close relationship to oestrone but without the acidic properties associated with a phenolic substance. It gives typical derivatives such as the acetate (m.p. 164.5 to 165.5) the oxime (m.p. 209 to 211) and the semicarbazone (m.p. 276). Its

structure was finally proved by a partial synthesis which was achieved by Ruzicka³ in 1934 by the oxidation of epicholestanyl acetate (see page 84). On oxidation androsterone gives androstandione which on reduction by Clemmensen's method with amalgamated zinc and hydrochloric acid gives the parent saturated hydrocarbon androstane.

Dehydrosoandrosterone ($C_{19}H_{28}O$) 3(β) Hydroxy 17 keto Δ^5 androstene. In 1934 Butenandt and Dannenbaum³¹ isolated a second substance from human male urine which proved to be a physiologically inactive unsaturated chloro ketone formed during the process of isolation by the action of hydrochloric acid on the corre-



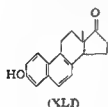
sponding hydroxy ketone. Treatment of the chloro compound with potassium benzoate followed by hydrolysis gave the hydroxy ketone which was also isolated from the urine in small yield and which was given the name dehydrosoandrosterone (XLIV). Like androsterone it is a hydroxy ketone but with a



confirmed by Marrian and Haslewood²³ Cohen Marrian and Odell²⁴ showed that oestriol was present in the pregnancy urine in the form of a glycoside with glucuronic acid the glycosidic link involving the secondary hydroxyl group at position 16 in the *cyclopentane* ring Oestriol has also been found in female willow catkins²⁵

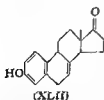
This hormone is only about one fifth as active as oestrone and in contrast to oestrone it is able to penetrate the intestinal membranes and is only slightly less active when administered by mouth than when injected Oestriol gives rise to a series of esters and the tri acetate (m p 127) is said to be about ten times more active than the free triol Oestriol can be prepared from oestrone (see page 81)

(+) Equilenin ($C_{18}H_{18}O$) 3 Hydroxy 17 keto- $\Delta^{1,3,5,8}$ oestrapentaene Using the newly discovered Girard Reagent T for the isolation and purification of the ketonic constituents of the urine of pregnant mares, Girard and his collaborators in 1932 and 1933²⁶ isolated two unsaturated ketones to which the names (+) equilenin and equilin were given Equilin contains two atoms of hydrogen less than oestrone and equilenin four atoms of hydrogen less (+) Equilenin



(XLI) has in fact the properties of a naphthalene derivative since rings A and B are both aromatic in character It gives rise to esters and ethers such as the acetate (m p 156 to 157°) benzoate (m p 222 to 223) and methyl ether (m p 197 to 198) Its structure has been established by degradation and by total synthesis (see page 82)

Equilin ($C_{18}H_{16}O_2$) 3 Hydroxy 17 keto $\Delta^{1,3,5,7}$ oestratetraene This hormone was isolated together with (+) equilenin by Girard and his



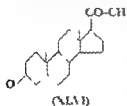
collaborators in their work on the urine of pregnant mares Equilin (XLII) has not been synthesised but it can be converted by treatment with palladium in nitrogen to (+) equilenin It gives rise to esters and ethers such as the benzoate (m p 197 to 198) and the methyl ether (m p 160.5 to 161.5)

Androgens

Isolation Androsterone may be extracted from male urine by methods similar to those used in the extraction of oestrone from pregnancy urine but since androsterone is not phenolic in type it is found in the neutral portion of the extract In the earlier method of Butenandt the urine was made acid concentrated and extracted with chloroform after

cent) and extracting with small volumes of light petroleum to remove impurities. After dilution the alcoholic solution is again extracted with light petroleum which removes the progesterone and leaves the estrone in the aqueous alcohol. Crystalline progesterone is obtained from the residue from the final light petroleum extract by trituration with ether in the cold. The crude progesterone can be crystallised from dilute alcohol or pyridine.

Progesterone ($C_{21}H_{30}O$) Δ^4 Pregnene 3,20 dione. After the ripening and rupture of the follicle in the human ovary a yellow tissue known as the corpus luteum is formed and is responsible for the secretion of progesterone (XLVI). Butenandt^{36, 37, 38} reported the isolation of the hormone in a pure crystalline form in 1934 and at about the same time similar reports were made independently by Slotta, Ruschig and Fels³⁹ by Allen and Wintersteiner⁴⁰ and by Hartmann and



Wittstein⁴¹ although these early reports were somewhat confused owing to the existence of polymorphic forms melting at 121 and 128 respectively. Progesterone is a diketone containing an $\alpha\beta$ double bond. It has been synthesised by numerous methods from cholesterol and stigmasterol (see page 89).

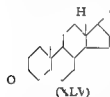
ADRENAL CORTICAL HORMONES

The hormones of the adrenal cortex are closely related chemically to the sex hormones. Some twenty-eight crystalline compounds of the steroid series have been isolated from extracts of the adrenal cortex.

Isolation. The separation of the medulla from the cortex is not practicable on a large scale. The whole glands are therefore extracted with alcohol at room temperature and the alcohol is removed by distillation. The residue is taken up in benzene and then in acetone and after partition between alcohol (70 per cent) and *n*-hexane the alcohol layer is filtered through a column of Permunt to remove the adrenaline. After evaporation the residue is taken up in water to eliminate resinous material. After a further precipitation from alcoholic solution an active dry extract is obtained^{42, 43}. In an alternative method the glands are extracted with acetone and after two partitions between alcohol (70 per cent) and light petroleum the material is extracted with ethylene dichloride⁴⁴. The active products are soluble both in water and in ether and benzene and the further purification of the hormones depends on repeated partition between solvents followed by the use of carbonyl reagents and chromatographic methods on both the free hormones and their acetates. The original publications must be consulted for the detailed procedures employed. Three groups of workers under Kendall, Wintersteiner and Reichstein have thus isolated a large number of

3(β) hydroxyl group and it contains a double bond between the carbon atoms at positions 5 and 6. It gives rise to an acetate (m p 171 to 172) an oxime (m p 188 to 189) and a semicarbazone acetate (m p 268). On oxidation it gives an androstendione in which the double bond has migrated into ring A. Its constitution was confirmed by its synthesis from cholesterol by oxidation of the acetate dibromide with chromic acid (see page 86)

Testosterone ($C_{19}H_{28}O_2$) 17 Hydroxy 3 keto Δ^4 androstene
Physiological tests carried out on extracts from male urine indicated the



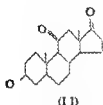
presence of one or more androgenic compounds of greater potency than either androsterone or dehydroandrosterone. This activity was found to be unstable towards treatment with hot alkalis which suggested the presence of an $\alpha\beta$ unsaturated ketone. In 1935 David Dingemans, Freud and Laqueur³⁰ reported the isolation of the new hormone (XLV) to which the name testosterone was given. It is an unsaturated hydroxy ketone with the same empirical formula as dehydroandrosterone but whereas the latter compound had the secondary alcohol group at position 3 and the ketone group at position 17 in testosterone the positions are reversed. Such a formulation is in agreement with its instability towards alkali which property is not shown by dehydroandrosterone which is not an $\alpha\beta$ unsaturated ketone. The structure was confirmed by its synthesis from dehydroandrosterone (see page 87). Testosterone gives rise to typical derivatives such as the acetate (m p 140 to 141) propionate (m p 121 to 123) benzoate (m p 198 to 200) and oxime (m p 222 to 223)

Progesterone

Isolation. To isolate progesterone the ovaries of sows or the corpora lutea are extracted with alcohol and after removal of the solvent by distillation the residue is dissolved in ether. The phospholipoids are precipitated with acetone cholesterol and fats are frozen out from solution in methyl alcohol and acidic compounds are removed with aqueous sodium bicarbonate solution. Estrone may be eliminated by partition between alcohol and light petroleum and the final purification is carried out by the use of a typical carbonyl reagent such as semicarbazide hydrochloride³⁴. The yield from 100 kilograms of ovaries is about 50 milligrams. In an improved procedure due to Allen and Goetsch³⁵ the minced ovaries are extracted with methyl alcohol and diluted with an equal volume of water to precipitate fatty substances. Subsequent extraction with light petroleum removes the progesterone which can be isolated in crude form on evaporation. Further purification is effected by redissolving the crude extract in alcohol (70 per

acid of the acetate of 17 hydroxycorticosterone⁵¹ Two partial syntheses from 3(α) acetoxy 11 ketobisnorcholan-ic acid and pregnane 3 21 diol 11 20 dione diacetate respectively have been reported (see page 97)

Adrenosterone ($C_{23}H_{34}O_3$) Δ^4 Androstene 3 11 17 trione This compound (II) (m p 222 $[\alpha]_D + 262$) was isolated by Reichstein⁵² from the portions of the adrenal extract which were more soluble in ether It possesses no adrenocortical hormone activity but shows a definite androgenic action On hydrogenation it gives the saturated triketone which on reduction by the Clemmensen method gives the parent hydrocarbon androstane

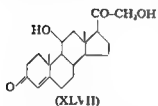


SYNTHESIS OF THE STEROID HORMONES

The synthesis of the various steroid hormones constitutes a formidable problem because the reduced cyclopentenophenanthrene system can exist in a large number of different stereoisomeric configurations and most synthetic processes will give rise to a complex mixture of stereoisomerides rather than to the one particular variety found in nature There is abundant evidence that in this series of compounds biological activity is intimately related to stereochemical configuration and it is therefore essential that any synthetic products prepared for use in medicine should possess the precise stereochemical structure found in the natural product In the testosterone and progesterone molecules there are eight asymmetric carbon atoms which implies the existence of a total of 256 stereoisomeric forms In the oestrone molecule which possesses one aromatic ring the number of asymmetric carbon atoms is reduced to four which implies only sixteen stereoisomeric configurations whereas in the equilenin molecule with two aromatic rings there are only two asymmetric carbon atoms and four stereoisomeric forms It is therefore not surprising that of all the steroid hormones equilenin was the first to yield to a total synthesis and more recently this has been followed by a total synthesis of oestrone which also implies the total synthesis of oestradiol and oestriol No total synthesis has yet been reported of testosterone progesterone or any of the hormones of the adrenal cortex although recent developments in particular the synthesis by Cornforth and Robinson⁵³ of a tricyclic ketone containing sixteen carbon atoms united in the precise stereochemical form found in coprostanone indicate that total syntheses of these and other similar hormones seem likely of achievement in the near future Numerous partial syntheses many of which have been developed into commercial processes have however been reported for dehydroandrosterone

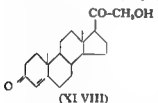
pure steroid compounds some of which are biologically active and others inactive. The more important members are corticosterone, deoxycortone, dehydrocorticosterone, cortisone, and adrenosterone. Progesterone, Δ^4 androstene-3,17-dione and oestrone have also been isolated by this method.

Corticosterone ($C_{21}H_{30}O_4$) Δ^4 Pregnene-11,21-diol-3,20-dione



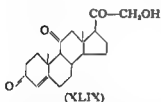
Corticosterone (XLVII) (m.p. 180 to 182, $[\alpha]_D^{25} + 223$ in alcohol) has a constitution similar to that of progesterone but with hydroxyl groups attached to the carbon atoms at positions 11 and 21.⁴⁵ The physiological activity is associated mainly with the hydroxyl group at position 21.

Deoxycortone ($C_{21}H_{28}O_3$) Δ^4 Pregnene-21-ol-3,20-dione



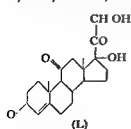
Deoxycortone (XVIII) (m.p. 141 to 142, $[\alpha]_D^{25} + 178$) differs from corticosterone by the absence of the hydroxyl group from position 11. It is more active than corticosterone and has been synthesised from dehydroandrosterone and from 3-hydroxycholesterol (see page 95).

Dehydrocorticosterone ($C_{21}H_{26}O_4$) Δ^4 Pregnene-21-ol-3,11,20-trione

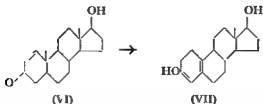


Dehydrocorticosterone (XLIX) (m.p. 174 to 181.5, $[\alpha]_D^{25} + 299$) was isolated by Mason, Myers and Kendall⁴⁶ and it has also been prepared by Reichstein by the oxidation of corticosterone acetate followed by hydrolysis.

Cortisone ($C_{21}H_{28}O_5$) Δ^4 Pregnene-17,21-diol-3,11,20-trione

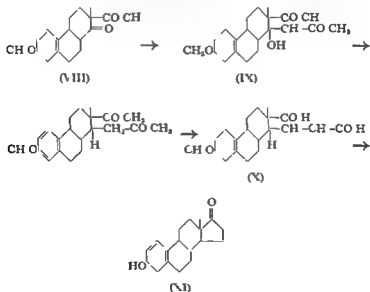


Cortisone (L) (m.p. 215, $[\alpha]_D^{25} + 209$) to which considerable attention has recently been drawn as a result of reports of its successful use in the treatment of rheumatic diseases, has been isolated in four different laboratories.^{46, 47, 48, 49, 50} The hydroxyl group at position 17 has the (α) configuration. The hormone has also been obtained by oxidation with chromic



from cholesterol have been investigated and confirmed by independent workers⁵⁷⁻⁵⁸. This conversion of an androgenic hormone to an oestrogenic hormone proves that both types possess the same basic stereochemical features.

In the total synthesis of oestrone due to Anner and Miescher⁵⁹ one of the crystalline racemic modifications of the keto ester (VIII) previously prepared by Robinson and Walker⁶⁰ and by Bachmann, Kushner and Stevenson⁶¹ as a liquid mixture of racemates is subjected to a Reformatsky reaction with methyl bromoacetate which gives four isomeric dicarboxylic esters (IX) which are then dehydrated and reduced. By means of half hydrolysis and the applications of the Arndt-Eistert homologation reaction the acid (X) is obtained which on cyclisation and decarboxylation gives racemic oestrone (XI) which is subsequently resolved to give (+) oestrone identical with the natural product.

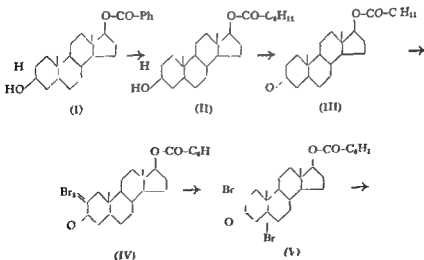


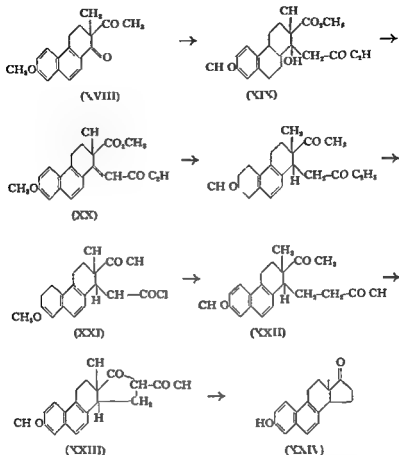
The conversion of oestrone to oestriol in an overall yield of 25 per cent has been reported⁶. The methyl ether of oestrone (XII) is converted by treatment with isomyl nitrite in tertiary butyl alcohol into

testosterone progesterone and deoxycortone the processes use a sterol such as cholesterol or stigmasterol as the starting product. By using such sterols in this way the correct stereochemical configuration for the final hormone is in the main already pre formed and the somewhat limited chemical changes involved in the synthesis can usually be made in such a way that the stereochemical configuration is controlled. More recently it has been shown that certain sapogenins can be used as starting materials for the preparation of various steroid hormones.

Oestrone Oestradiol and Oestriol Two methods are available for the synthesis of oestrone and hence of oestradiol and oestriol.

In the Inhoffen^{54 55} process Δ^5 androstene 3(β) 17(α) diol 17 benzoate (I) an intermediate obtained in the synthesis of testosterone from cholesterol is reduced catalytically to androstanediol 17 hexahydrobenzoate (II), which on oxidation with chromic acid gives androstanolone 17 hexahydrobenzoate (III). Bromination in acetic acid solution gives 2,2 dibromoandrostanolone 17 hexahydrobenzoate (IV) which then undergoes rearrangement in the presence of hydrogen bromide in acetic acid to 2,4 dibromoandrostanolone 17 hexahydrobenzoate (V). Removal of hydrogen bromide with collidine followed by hydrolysis gives the androstadienolone (VI). A solution of this compound in tetralin is then blown in a spray through a quartz tube maintained at 600° in an atmosphere of nitrogen and this results in the aromatisation of ring A and the expulsion of the angular methyl group. The oestradiol (VII) thus formed is isolated in the form of a crystalline complex with tetralin from which the free oestradiol is obtained by distillation in steam.⁵⁶ The essential stages in this synthesis of oestradiol

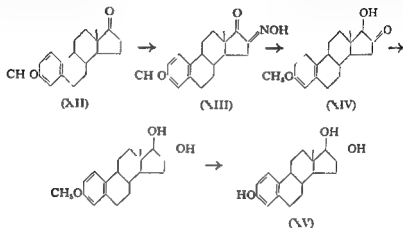




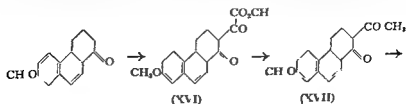
the half ester (XXI) and then the chain was lengthened by the Arndt Eistert procedure to give the ester (XXII) of the corresponding propionic acid cyclisation of which gave 16 carbomethoxy \pm equilenin methyl ether (XXIII). The removal of the carbomethoxy group and the methyl group was effected by heating with acetic acid and hydrochloric acid and the resulting racemic equilenin (XXIV) was resolved by forming α -menthoxyacetic esters. In this manner (+) equilenin was obtained which was shown to be identical with the natural product together with (–)-equilenin. Resolution of the product from the second racemic saturated acid gave (+) and (–) *isoequilenin*.

In a more recent synthesis⁶⁴ 1-keto-7-methoxy-1,2,3,4-tetrahydrophenanthrene is converted by treatment with ethyl formate and sodium methoxide into (XXV) which with hydroxylamine gives the *iso*-oxazole (XXVI). This is converted by methylation into the cyano-ketone (XXVII) which on condensation with methyl succinate in the

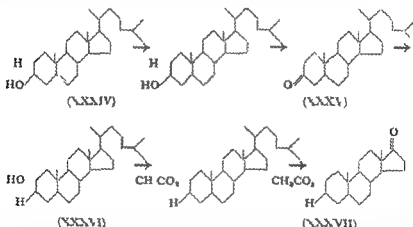
16 *iso* nitroso œstrone methyl ether (XIII) which on boiling with zinc dust in acetic acid solution gives 16 hydroxyœstrone methyl ether (XIV). Reduction with sodium and *iso*propyl alcohol converts the carbonyl group into a secondary alcohol group and subsequent demethylation with a mixture of hydrobromic acid and acetic acid gives œstriol (XV).



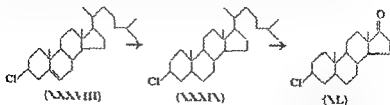
Equilenin Equilenin one of the female hormones found in the urine of pregnant mares was the first sex hormone to be synthesised completely. This was achieved by Bachmann, Cole and Wilds⁶³ in 1940 who obtained the four stereoisomerides, one of which was shown to be identical with the natural product. By the action of methyl oxalate on 7-methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene they obtained the glyoxalate (XVI) which on heating lost carbon monoxide to give 7-methoxy-1-keto-2-carbomethoxy-1,2,3,4-tetrahydrophenanthrene (XVII) in good yield. As a β -ketonic ester this compound reacted with methyl iodide to give 7-methoxy-1-keto-2-methyl-2-carbomethoxy-1,2,3,4-tetrahydrophenanthrene (XVIII). This compound was then subjected to a Reformatsky reaction with ethyl bromoacetate to give the hydroxy ester (XIX) which in turn was converted to a mixture of the two geometrically isomeric unsaturated esters (XX). Reduction of the double bond gave a mixture of the four possible isomeric saturated acids which formed two racemates which were separated by crystallisation. Each racemic acid was in turn converted to the acid chloride of



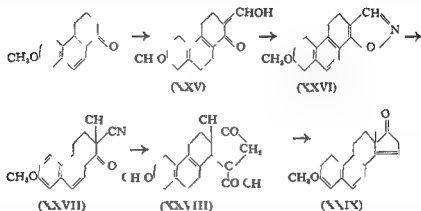
This method of synthesis indicates that androsterone belongs to the cholestane or *allo* series and is an alcohol of the *epi* type. The yields obtained in this oxidation are very small and Callow and Deanesly⁶⁵ using an improved process were able to obtain an overall yield of only 0.2 per cent. In this process cholesterol (XXXIV) is first reduced with hydrogen over a platinum catalyst to give dihydrocholesterol which on oxidation with chromic acid gives cholestanone (XXXV). Catalytic reduction of cholestanone with hydrogen in acid solution gives *epi*dihydrocholesterol (XXXVI) which is converted to the acetate and then subjected to oxidation with chromic acid under more drastic conditions. The androsterone acetate (XXXVII) thus formed is isolated from the mixture of oxidation products and unchanged *epi*dihydrocholesteryl acetate either by formation of the semicarbazone or with the use of a Girard reagent. The free androsterone (XXXIII) is then isolated by hydrolysis.



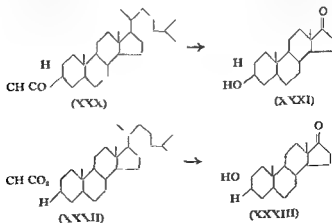
An improved synthesis of androsterone has been described by Marker^{66, 67} who converted cholesterol (XXXIV) to cholesteryl chloride (XXXVIII) by treatment with phosphorus pentachloride and then reduced the chloride catalytically to give (α) cholestyl chloride (XXXIX). Oxidation of which gave (α) chloroandrosterone (XL) which was hydrolysed to androsterone (XXXIII).

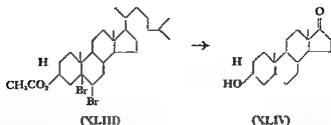


presence of potassium *tert* butoxide affords 15 carbomethoxy 14 15 dehydroequilenin methyl ether (XXVIII). Hydrolysis to the free acid followed by decarboxylation gives the methyl ether of 14 15 dehydroequilenin (XXIX) which on hydrogenation and demethylation gives racemic equilenin (XXXIV) from which the natural hormone can be obtained by resolution as before



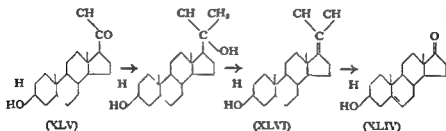
Androsterone Ruzicka showed that the oxidation of cholesteryl acetate (dihydrocholesteryl acetate) (XXX) with chromic anhydride in hot glacial acetic acid solution gave a small neutral fraction which proved to be 3 hydroxycholestan-17 one or *iso*androsterone (XXXI) a less active stereoisomer of the androsterone isolated from natural sources. In order to obtain the correct stereochemical configuration Ruzicka⁸ applied the same method of oxidation to *epi*dihydrocholesteryl acetate (XXXII) as well as to coprosteryl acetate and *epi*coprosteryl acetate. The ketone (XXXIII) derived from *epi*dihydrocholesteryl acetate proved to be identical with androsterone





A similar method of synthesis was reported by Oppenauer⁷¹ who used sitosterol obtained from soya beans in place of cholesterol and Butenandt, Dannenbaum, Hanisch and Kudzusz⁷² described the preparation of dehydroisoandrosterone from stigmasterol as well as from cholesterol. In their preparation from cholesterol by the above method a yield of 2% per cent. was reported. Full details of the process for the oxidation of cholesteryl acetate dibromide to dehydroisoandrosterone acetate have been published⁷⁴.

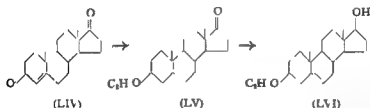
Using various sapogenins as starting materials Marker and his co-workers⁷ have evolved a new method for the preparation of pregnenolone (XLV) which provides indirectly a new route to dehydroisoandrosterone (XLIV). When treated with methylmagnesium halides pregnenolone gives a tertiary alcohol which loses water to give the isopropenyl derivative (XLVI) from which dehydroisoandrosterone (XLIV) is obtained on ozonolysis.



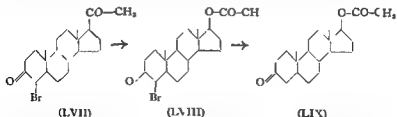
Testosterone The synthesis of testosterone was first accomplished almost simultaneously by Ruzicka^{73, 74} and by Butenandt.⁷ Dehydroisoandrosterone (XLIV) obtained from cholesterol was reduced with sodium and propyl alcohol to androstene 3, 17-diol (XLVII) and then converted to the diacetate (XLVIII). Partial hydrolysis of the diacetate gave 17-acetoxyandrostene 3-ol (XLIX) which was brominated at the double bond and oxidised to convert the hydroxyl group at position 3 to a ketone group. Removal of the bromine atoms and hydrolysis then gave testosterone (4⁴ androstene 17(β)-ol 3-one) (L) identical with the hormone isolated from male urine.

Later modifications were introduced whereby the reduction of dehydroisandrosterone acetate to 3-acetoxyandrost-17-ol was effected catalytically with Raney nickel in methyl alcoholic solution and the oxidation of the androstendiol benzoate to testosterone benzoate was carried out with aluminium isopropoxide and cyclohexanone in the presence of toluene. For clinical purposes the testosterone thus produced is converted into the propionate. Full details of this process have been published⁵⁶. The overall yield of testosterone propionate from cholesterol is 2.4 per cent.

An alternative synthetic route to testosterone from dehydroisandrosterone involves the oxidation of the latter to androstendione (LIV) which may then be converted into the 3-enol ethyl ether (LV). The ketone group at position 17 in the enol ether is reduced to the secondary alcohol to give a mixture of the 3-enol ethers of the two isomeric androst-17-ol-3-ones (LVI) which gives testosterone (L) and an isomeride on hydrolysis during which the free enol form reverts to the keto form⁷⁷.

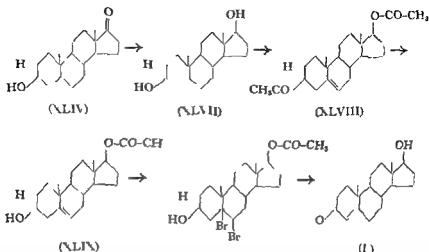


A new method for the synthesis of testosterone using sapogenins as starting materials has been reported by Marker⁷⁸. Oxidation of pseudo-sarsapogenin leads to pregnane-3,20-dione and persulphate oxidation of 4-bromopregnane-3,20-dione (LVII) in acetic acid produces the 17-acetoxy compound (LVIII) which loses hydrogen bromide on treatment with pyridine to give testosterone acetate (LIX).



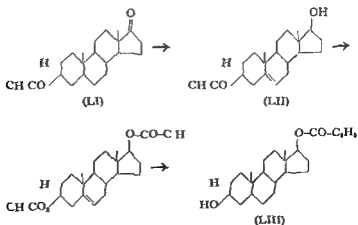
Progesterone Fernholz⁷⁹ showed in 1933 that stigmasteryl acetate can be converted into the 5,6-dibromide (LX) which when submitted to ozonolysis and debromination gave Δ^5 -3-acetoxybisanorcholenic acid (LXI). An ester of this acid was converted by Butenandt, Westphal and Cobler⁸⁰ through the diphenyl carbinol into the unsaturated

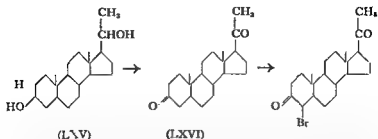
HORMONES



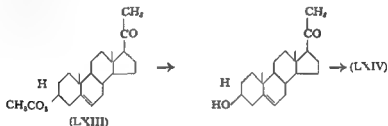
In the reduction of dehydro α androsterone to androstendiol a new asymmetric centre has been created and the product will therefore consist of a mixture of two stereoisomeric androstendiols both of which are capable of conversion to the corresponding androsten 17 ol 3 one. In the synthesis of testosterone it is therefore necessary to separate the correct stereochemical variety of androstendiol. The second isomeride leads to the less active androsten 17(α) ol 3 one.

An important improvement in the above synthesis was subsequently reported by Ruzicka, Wettstein and Kagi⁷⁶ who reduced dehydro α androsterone acetate (LI) to 3 acetoxyandrosten 17 ol (LII) and converted the latter to the 17 benzoate which mixed ester proved to be more suitable for partial hydrolysis than the 3 17 diacetate. The resulting 17 benzoyloxyandrosten 3 ol (LIII) was then oxidised and debenzoylated to give testosterone (L).

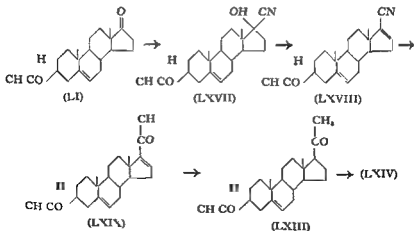




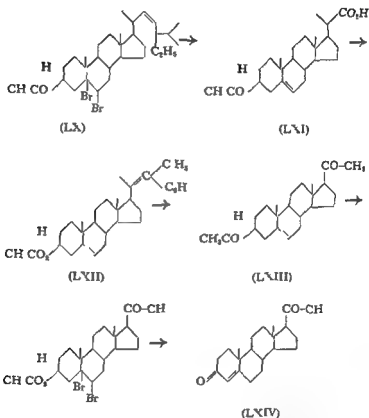
Progesterone may also be synthesised from Δ^5 pregnen 3(β) ol 20 one acetate (LXIII) a by product from the chromic acid oxidation of cholesteryl acetate dibromide to dehydroisandrosterone by hydrolysis and oxidation⁸⁴



Butenandt⁸⁵ has described a synthesis of progesterone from dehydroisandrosterone acetate (LI) which on treatment with hydrogen cyanide gives the cyanhydrin (LXVII) which is then dehydrated to the nitrile (LXVIII). The unsaturated nitrile reacts with methylmagnesium bromide to give the ketone (LXIX) which is converted to pregnenolone acetate (LXIII) on reduction. The pregnenolone acetate obtained in this way is converted into progesterone (LXIV) as described above

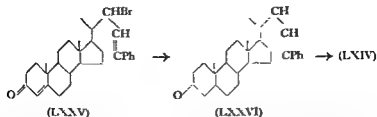


compound (LXII) Protection of the double bond between positions 5 and 6 by the addition of bromine followed by oxidation debromination and hydrolysis gave the acetate of Δ^5 pregnen 3(β) ol 20 one (LXIII) This compound after protection of the double bond by bromination was then oxidised and subsequent debromination gave progesterone (Δ^4 pregnen 3 20 dione) (LXIV) identical with the natural product



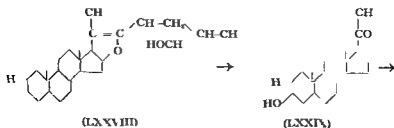
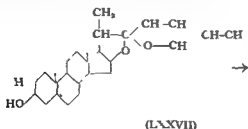
In the removal of the bromine with zinc dust the double bond is not regenerated at the original 5 6 position but appears at the 4 5 position in conjugation with the ketone group at the 3 position. A similar synthesis was reported independently by Fernholz⁶¹ Details of this process with many improvements have been published⁶⁶

Butenandt⁶² also prepared progesterone from pregnane 3 20 diol (LXV) a reduction product of progesterone isolated from human pregnancy urine. In one such method it was oxidised to pregnanedione (LXVI), which on bromination and subsequent treatment with pyridine to remove hydrogen bromide gave progesterone (LXIV)

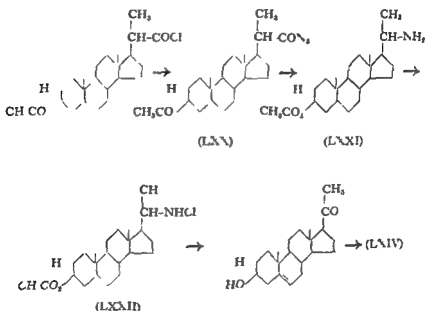


followed by treatment with *N* bromosuccinimide affords the bromo-compound (LXXV) which on loss of hydrogen bromide gives the ketone (LXXVI). This on oxidation yields progesterone (LXIV). The method provides an interesting example of the use of *N* bromosuccinimide which effects the removal of three carbon atoms in the side chain in one stage.

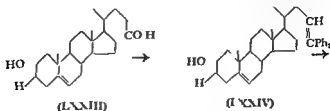
An entirely new synthetic route to progesterone from certain saponins has been provided by the investigations of Marker⁸⁷. Diosgenin (LXXVII) obtained from trillin, a glycoside in the root of *Trillium erectum*, may be isomerised by heating with acetic anhydride at 200° to the pseudo form (LXXVIII) which on mild oxidation with chromic acid gives mainly Δ^5 pregnadien-3(β)-ol-20-one (LXXIX). Hydrogenation of the latter over a palladium barium sulphate catalyst gives Δ^5 pregnen-3(β)-ol-20-one (LXXX) which can then be converted to progesterone (LXIV) by oxidation. Alternative routes from diosgenin isolated from *Dioscorea tokoro* (Makino) have also been reported⁸⁸. Sarsasapogenin has similarly been used for the synthesis of pregnan-3,20-diol⁸⁹ (LXV).



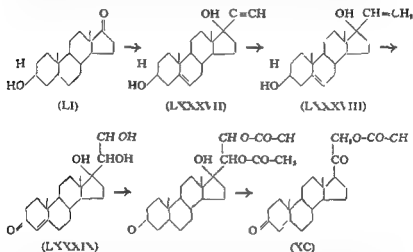
The degradation of Δ^5 3 acetoxybisorcholenic acid to progesterone on a commercial scale was also achieved by Ruschig who converted the acid to the acid chloride and thence to the azide (LXX) The latter evolved nitrogen when heated and gave the isocyanate which was converted to the amine (LXXI) on hydrolysis The amine was converted to the chloramine (LXXII) with hypochlorous acid and treatment with sodium ethoxide gave the ketimine, which was readily hydrolysed to pregnenolone This can be converted to progesterone (LXIV) by oxidation with aluminium *tert* butoxide in the presence of acetone and benzene⁶⁴



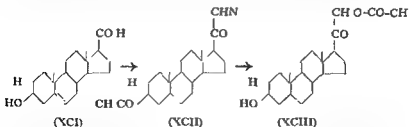
A number of new synthetic routes to progesterone have recently been reported by Meystre Wettstein and Miescher⁶⁵ In one of these Δ^5 3(α) hydroxybisorcholenic acid (LXXIII) is converted through its methyl ester and reaction with phenyl magnesium bromide into the diphenylethylene (LXXIV) Oxidation of the hydroxyl group



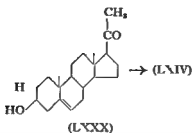
Deoxycortone The conversion of dehydroandrosterone (LI) into deoxycortone was achieved in 1939 by Serini Logemann and Hildebrand⁹² who first prepared the addition compound with acetylene (LXXVII) namely ethynyl androsten 3 17 diol which was then partly reduced catalytically to the corresponding vinyl compound (LXXVIII) After oxidation of the hydroxyl group at position 3 the vinyl group was converted to the glycol (LXXIX) and then to the di acetate from which one molecular proportion of acetic acid was removed on heating with zinc dust to give deoxycortone acetate (XC) by molecular rearrangement Deoxycortone acetate can be



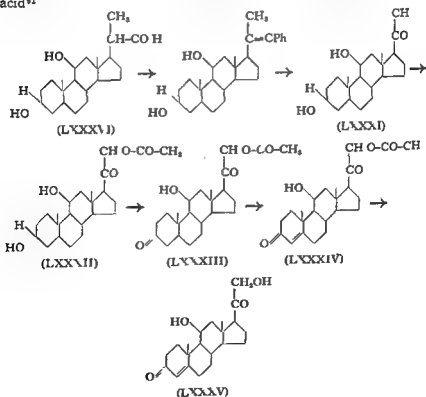
more conveniently prepared from 3 hydroxy Δ^5 ætiocholenic acid (XCI) a by product from the chromic acid oxidation of cholesteryl acetate dibromide which after acetylation is converted to the acid



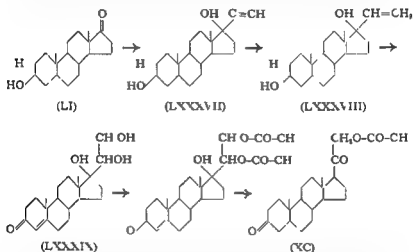
chloride and then treated with diazomethane to give the diazo ketone (XCII) After hydrolysis and treatment with acetic acid nitrogen is evolved and the pregnenolone acetate (XCIII) is formed which may then be oxidised by the Oppenauer method to give deoxycortone acetate^{95 97} = (XC)



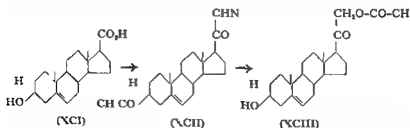
Corticosterone The partial synthesis of corticosterone has been reported by von Euw Lardon and Reichstein⁹⁰ The action of lead tetra acetate on pregnane 3 11 diol 20 one (LXXXI) gave pregnane 3 11 21 triol 20 one 21 acetate (LXXXII) which on oxidation with aluminium phenoxide in acetone afforded pregnane 11 21 diol 3 20 dione 21 acetate (LXXXIII) Bromination in acetic acid solution and subsequent dehydrobromination with pyridine yielded corticosterone acetate (LXXXIV) which was hydrolysed to give corticosterone (LXXXV) The pregnane 3 11 diol 20 one (LXXXI) was obtained from the methyl ester of the appropriate 3 11 dihydroxybisanorcholanic acid (LXXXVI) which in turn was obtained from bisnordeoxycholic acid⁹¹



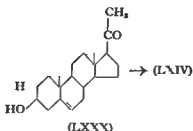
Deoxycortone The conversion of dehydroandrosterone (LI) into deoxycortone was achieved in 1939 by Serini Logemann and Hildebrand⁹ who first prepared the addition compound with acetylene (LXXVII) namely ethynyl androsten 3 17 diol which was then partly reduced catalytically to the corresponding vinyl compound (LXXVIII). After oxidation of the hydroxyl group at position 3 the vinyl group was converted to the glycol (LXXIX) and then to the diacetate from which one molecular proportion of acetic acid was removed on heating with zinc dust to give deoxycortone acetate (XC) by molecular rearrangement. Deoxycortone acetate can be



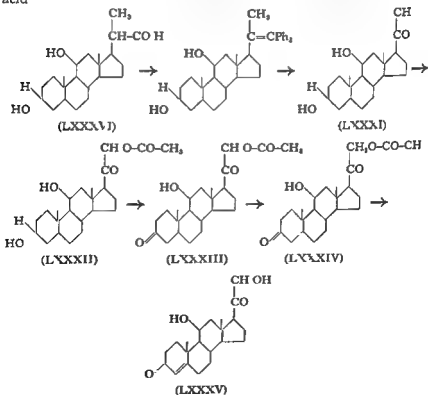
more conveniently prepared from 3 hydroxy Δ^4 aetiocholenic acid (XCI) a by product from the chromic acid oxidation of cholesteryl acetate dibromide which after acetylation is converted to the acid

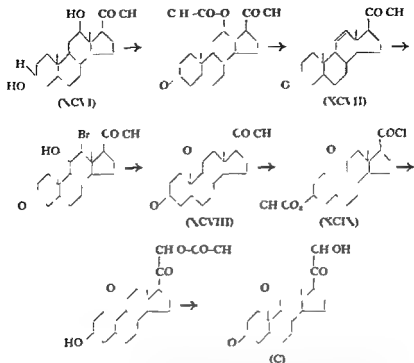


chloride and then treated with diazomethane to give the diazo ketone (XCII). After hydrolysis and treatment with acetic acid nitrogen is evolved and the pregnen-20-one-3 α -ol-20-one acetate (XCIII) is formed which may then be oxidised by the Oppenauer method to give deoxycortone acetate^{56 93 94} (XC).

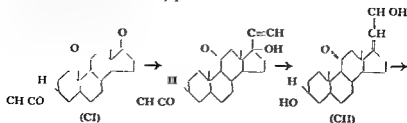


Corticosterone The partial synthesis of corticosterone has been reported by von Ew, Lardon and Reichstein⁹⁰ The action of lead tetra acetate on pregnane 3 11 diol 20 one (LXXXI) gave pregnane 3 11 21 triol 20 one 21 acetate (LXXXII) which on oxidation with aluminium phenoxide in acetone afforded pregnane 11 21 diol 3 20 dione 21 acetate (LXXXIII) Bromination in acetic acid solution and subsequent dehydrobromination with pyridine yielded corticosterone acetate (LXXXIV) which was hydrolysed to give corticosterone (LXXXV) The pregnane 3 11 diol 20 one (LXXXI) was obtained from the methyl ester of the appropriate 3 11 dihydroxybisorcholic acid (LXXXVI) which in turn was obtained from bisnordeoxycholic acid⁹¹

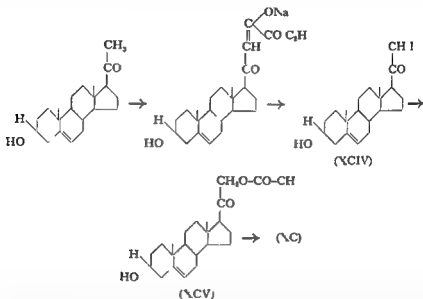




Cortisone Two methods for the synthesis of this compound have been reported by Sarett. In his first method¹⁶ the diketone (CI) was condensed with acetylene and then subjected to partial reduction with a subsequent allylic rearrangement to give (CII). The 21 hydroxyl group was protected by succinylation after which the hydroxyl group at position 3 was oxidised to give (CIII) which was treated with acetic anhydride and osmium tetroxide in order to hydroxylate the double bond. The resulting compound (CIV) was treated successively with bromine and pyridine to introduce the double bond into ring A and the diacetate (CV) was hydrolysed and partially reacylated to give the 21 monoacetate (CVI) which was then cautiously oxidised at the 20-hydroxyl group to give cortisone acetate (CVII). Adrenosterone was obtained as a by product.



Deoxycortone acetate (XC) can also be obtained from pregnenolone obtained from 3 acetoxy Δ^5 bisnorcholeic acid by condensation with ethyl oxalate followed by hydrolysis of the resulting ester and treatment of the acid with iodine and alkali. The iodo compound (XCIV) is then boiled with potassium acetate and acetone this gives 21 acetoxypregnenolone (XCV) which is oxidised with aluminium *tert* butoxide in the presence of cyclohexanone and toluene to



deoxycortone acetate⁵⁶ (XC). Most of the other steroids isolated from the adrenal cortex contain either a keto or a hydroxyl group at position 11 and for these purposes cholesterol is not a suitable starting material. Deoxycholeic acid however has been successfully used as a starting material for the preparation of compounds for use in the synthesis of dehydrocorticosterone and cortisone.

Dehydrocorticosterone In the synthesis of this substance the methyl ester of bisnordeoxycholeic acid (XCVI) is oxidised and dehydrated by pyrolysis of the 12 benzoate to give the ester (XCVII). Addition of hypobromous acid at the double bond followed by oxidation and debromination gives the 3,11 diketone ester (XCVIII). The 3 keto group is reduced to the secondary alcohol the acetate of which is then converted to the acid chloride (XCIX). This is subsequently treated with diazomethane as in the synthesis of deoxycorticosterone from 3 hydroxy Δ^5 choleic acid. Finally after oxidation the double bond is introduced into ring A by bromination and subsequent elimination of hydrogen bromide with pyridine which gives dehydrocorticosterone (C) on hydrolysis⁵⁵.

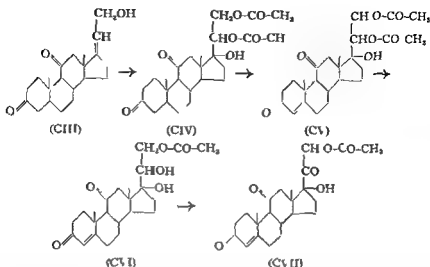
Improved methods of synthesis which avoid the use of the expensive osmium tetroxide have been outlined by Kendall⁹⁹. Sapogenins such as botogenin and the cardiac active principle sarmientogenin have also been suggested as raw materials for possible syntheses of cortisone. Sarmientogenin obtained from the seeds of the tropical vine *Strophan thus sarmientosus* possesses the great advantage of having an hydroxyl group attached to position 11.

ARTIFICIAL HORMONES

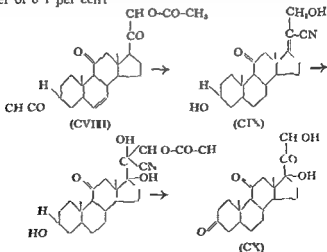
In the preceding pages it has been shown that most of the natural hormones have now been synthesised in the laboratory. Many of these syntheses are in reality partial syntheses since they depend on a sterol, bile acid or sapogenin as the starting material but in a few cases a total synthesis has been achieved as for example with equilenin and oestrone. A large number of artificial hormones or compounds possessing hormone like action have been synthesised in the laboratory. These belong mainly to the oestrogen series since the oestrogenic response is perhaps the least specific of all hormone reactions. They have been referred to as synthetic oestrogens but artificial oestrogens is a more appropriate term. These compounds are not found in nature and mostly they differ from the natural hormones in that they retain their activity when administered by mouth. A comprehensive review of the chemistry of the artificial oestrogens and the relation between structure and activity has been published by Solmsen¹⁰⁰.

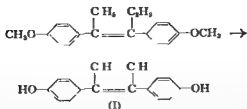
The fact that five different oestrogenic compounds had been isolated from natural sources namely (+)-oestrone, α -oestradiol, oestriol, equilin and (+)-equilenin suggested that the physiological reactions associated with oestrus did not involve a high degree of specificity. This observation led Dodds and his collaborators to look for synthetic compounds of simple structure which would be capable of reproducing all the known biological reactions of the natural oestrogenic hormones. Among the earlier compounds investigated was 1 keto-1,2,3,4-tetrahydrophenanthrene which showed positive oestrogenic activity of a low order of potency. Subsequently a large number of oestrogenically active substances were prepared some of which such as 9,10-dihydroxy-9,10-dinpropyl-1,10-dihydro-1,2,5,6-dibenzanthracene were found to be almost equal to oestriol in activity¹⁰¹. It was also demonstrated that the presence of the phenanthrene nucleus was not essential for oestrogenic activity and that a positive response was obtained even with such simple molecules as 4,4'-dihydroxydiphenyl, 4,4'-dihydroxydiphenylmethane, 4,4'-dihydroxydibenzyl and 4,4'-dihydroxystilbene¹⁰².

Further elaboration of the stilbene molecule led to the preparation by Dodds, Golberg, Lawson and Robinson¹⁰³ of the highly active 4,4'-dihydroxydiethylstilbene (I) generally known as stilboestrol or diethylstilboestrol. This compound is more active than oestrone when

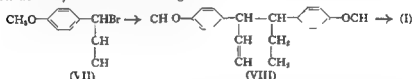


In the second synthesis⁹⁷⁻⁹⁸ pregnane 3,21-diol 11,20-dione diacetate (CVIII) was converted to the cyanhydrin and then dehydrated and hydrolysed to give (CIX). This was acetylated at the 21 position, hydroxylated with osmium tetroxide followed by treatment with aqueous sodium sulphite, oxidised at the 3 position and subsequently hydrolysed to give (CX). The 21 acetate was treated successively with bromine and boiling pyridine to introduce the double bond into ring A, thus giving the acetate of cortisone (CVII). The yields obtained in the final stages were subsequently improved by employing the method of Mattox and Kendall⁹⁹ for the dehydrobromination stage. In both methods of synthesis the ultimate starting material is deoxycholic acid. Some thirty consecutive stages are involved and the overall yield is of the order of 0.1 per cent.

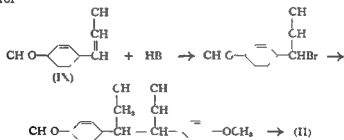




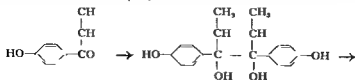
A more direct method of synthesis is due to Kharasch and Kleiman¹⁰⁶ who treated anethole hydrobromide (VII) with sodamide in liquid ammonia. The resulting product regarded as (VIII) gave stilbæstrol on demethylation and rearrangement



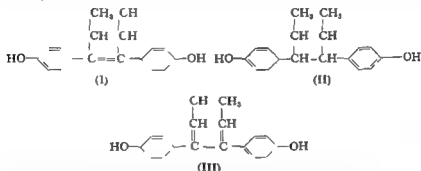
Hexæstrol(II) is prepared from anethole (IX) by addition of hydrogen bromide followed by a condensation of two molecules of the bromo compound in the presence of a metal such as sodium or magnesium. The resulting dimethyl ether of hexæstrol is demethylated with alcoholic potassium hydroxide or with a solution of hydrobromic acid in glacial acetic acid. The active product is the *meso* form of dihydro stilbæstrol^{106 107 108}



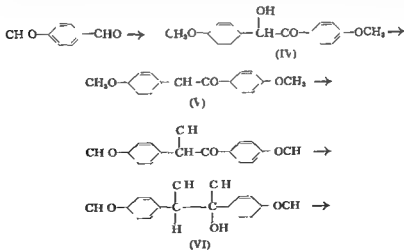
Dienæstrol (III) The preparation has been described by Dodds Golberg, Lawson and Robinson¹⁰³ who reduced *p* hydroxypropio phenone with an aluminium amalgam to the pinacol (X) which on dehydration with acetic anhydride and acetyl chloride gave the diacetyl derivative of dienæstrol. Hydrolysis with alcoholic potassium hydroxide gave dienæstrol (III)



administered subcutaneously and retains its activity when given by mouth. In an attempt to effect a further simplification of the molecule by the elimination of one of the hydroxyphenyl groups in stilboestrol anol (*p* hydroxypropenylbenzene) was investigated and found at first to be highly active but further investigation showed that this activity was due to an impurity which was later identified as 4,4 dihydroxy $\gamma\delta$ diphenyl *n* hexane (II) also known as hexoestrol which is a reduction product of stilboestrol¹⁰⁴. Another compound of similar type with high oral activity is 4,4 dihydroxy $\gamma\delta$ diphenylhexadiene or dien oestrol (III)

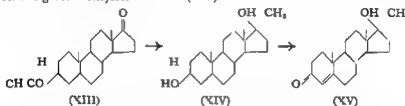


Stilboestrol (I) is prepared from anisaldehyde which undergoes a benzoin type condensation to give anisoin (IV). Reduction with stannous chloride gives deoxyanisoin (V) which is treated successively with ethyl iodide and ethylmagnesium bromide. The resulting tertiary alcohol (VI) is dehydrated to give the dimethyl ether of stilboestrol which is demethylated with alcoholic potassium hydroxide¹⁰⁵. The active product obtained in this manner has the *trans* configuration

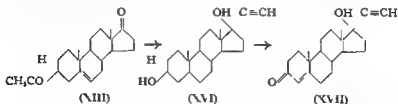


Steroid Derivatives Attempts have also been made to synthesize active compounds based on a steroid nucleus. Among such compounds may be mentioned methyltestosterone, ethisterone and ethinylœstradiol. Methyltestosterone which is not found in nature possesses male hormone activity. Ethisterone also is not a natural hormone but it shows the physiological reactions of progesterone. Ethinylœstradiol is said to be a slightly more active œstrogen than α œstradiol. Unlike the natural hormones all three compounds retain their activity when administered by mouth although at a somewhat lower level of activity.

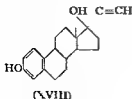
Methyltestosterone or 17 methyl Δ^4 androsten 17 ol 3 one (XV) is prepared from dehydroisandrosterone acetate (XIII) which on treatment with methyl magnesium bromide and subsequent hydrolysis gives 17 methylandrosterone 3 17 diol (XIV). Oxidation of the latter with aluminium isopropoxide in the presence of cyclohexanone and toluene gives methyltestosterone (XV).¹¹⁹

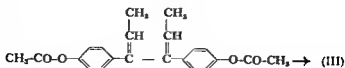


Ethisterone pregnenolone anhydrohydroxyprogesterone ethinyl testosterone or 17 ethinyl Δ^4 androsten 17 ol 3 one (XVII) is prepared from dehydroisandrosterone acetate (XIII) by treatment with potassium acetylide in liquid ammonia the resulting ethinyl androstendiol (XVI) being subjected to oxidation by the Oppenauer reaction.^{120 121 122}



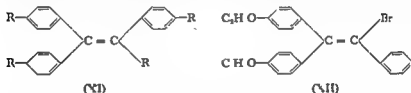
Ethinylœstradiol (XVIII) is obtained by the action of potassium acetylide on œstrone in liquid ammonia in much the same way that ethinyl androstendiol is obtained from dehydroisandrosterone.^{1 2}



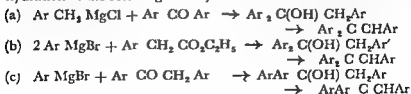


Subsequent improvements were introduced into this process by effecting the reduction to the pinacol with sodium amalgam or potassium amalgam in aqueous alkaline solution and by benzoylating the crude pinacol prior to dehydration and hydrolysis^{109 110} The chemistry of the artificial oestrogens of the stilboestrol type has been reviewed by Jones¹¹¹

Triphenylethylene Derivatives An entirely different group of artificial oestrogens is found in compounds of the triphenylethylene series Triphenylethylene (XI R=R=H) was shown to be highly active by Dodds¹² and independently by Robson and Schonberg¹¹² and subsequently improved properties were claimed for triphenyl chloroethylene (XI R=H R=Cl)¹¹⁴, tri *p* anisylbromoethylene (XI, R=OMe R=H)^{115 116} and β bromo β phenyl α di (*p* ethoxy phenyl) ethylene (DBE) (XII)¹¹⁷ Artificial oestrogens of this type are active by mouth and are characterised by having a prolonged action



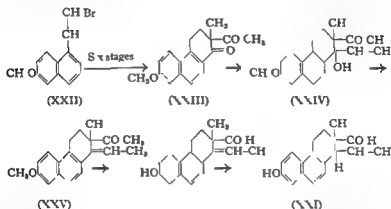
The preparation of artificial oestrogens of the triphenylethylene series can be readily effected either (a) by the action of a benzyl magnesium halide on a benzophenone (b) by the action of a phenyl magnesium halide on an ester of phenylacetic acid or (c) by the action of a phenyl magnesium halide on a deoxybenzoin with subsequent dehydration of the resulting tertiary alcohol in each case thus



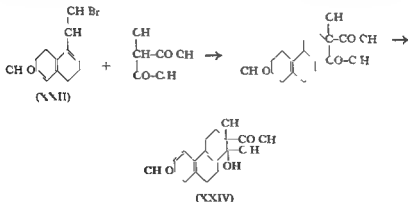
(Ar=an aryl group)

These reactions are capable of giving rise to a wide variety of substituted triphenylethylenes of various types and the corresponding chloro or bromo ethylenes may be obtained by the chlorination or bromination of either the carbinol or the ethylene¹¹⁸ In the former case the reaction is accompanied by loss of water

Bisdehydrodisynolic acid (XXI) and its methyl ether have been synthesised by Miescher¹²⁸ β (6 Methoxynaphthyl)ethyl bromide (XXII) was converted in six stages into the keto ester (XXIII) which was then treated with ethylmagnesium bromide. This keto ester (XXIII) was also encountered in Bachmann's synthesis of equilenin⁶³. The resulting carbinol (XXIV) was dehydrated with formic acid to give the ester (XXV) which on hydrolysis and reduction gave racemic bisdehydrodisynolic acid (XXI). This acid was subsequently resolved and the (—) acid was found to be identical with the acid prepared from equilenin.



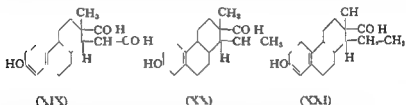
An alternative synthesis¹²⁹ utilises β (6 methoxynaphthyl)ethyl bromide (XXII) and methyl α propionylpropionate and proceeds by the following sequence of reactions giving the same carbinol (XXIV).



A detailed report on the stereochemistry and synthesis of these acids and of a large number of closely related compounds has been published by Shoppee¹³⁰

Doisyneic Acid and Derivatives A number of highly active oestrogenic acids have been obtained by the partial degradation of natural oestrogens on fusion with potassium hydroxide. The most active oestrogens at present known are found among these compounds the constitutions of which have been proved by total synthesis.

In 1932 Marrian and Haslewood^{1, 4} isolated a dicarboxylic acid ($C_{18}H_{20}O_6$) (XIX) since termed marrianolic acid by the fusion of oestriol with potassium hydroxide and shortly afterwards MacCorquodale, Levin, Thayer and Doisy^{1, 5} obtained a similar monocarboxylic acid ($C_{17}H_{18}O_5$) (XX) since termed doisyneic acid from similar treatment of (+) oestrone. More recently this latter acid has been obtained by Heer and Miescher in 50 per cent yield by fusion of (+) oestrone or oestradiol with potassium hydroxide at 275°.¹²⁶



While marrianolic acid (XIX) is inactive doisyneic acid (XX) is practically as active as (+) oestrone by subcutaneous administration. (–) Bisdehydrodoisyneic acid (XXI) obtained from (+) equilenin by fusion with potassium hydroxide¹²⁷ is even more potent being fully active in rats at a threshold dose of 0.05 microgram. The figures in Table X indicate the relationship between the activity of these compounds, the natural oestrogens and stilboestrol.

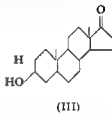
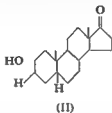
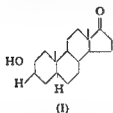
TABLE X
COMPARATIVE ACTIVITY OF SOME NATURAL AND ARTIFICIAL OESTROGENS

Compound	M.p.	[α] _D ²⁵ in ethyl alcohol	Oestrus threshold value by s.m. in dose (micrograms)	
			Subcutaneous	Oral
Bisdehydrodoisyneic acid (synthetic)	159 to 160	–116	0.05	0.05
(–) Bisdehydrodoisyneic acid (from equilenin)	161 to 162	–117	0.05	0.05
≡ Bisdehydrodoisyneic acid methyl ether	187	—	0.05	0.05
(+) Oestrone	259	+170*	0.7	20–30
α Oestradiol	176 to 178	+81	0.3–0.4	20–30
Stilboestrol	169 to 171	—	0.3–0.4	0.7–1.0

* in dioxan

excretion of both androgens and oestrogens and in particular of dehydroisoandrosterone

The main ketonic constituents of normal male and female urine are androsterone (I) 3 hydroxyaetiocholan 17 one (II) and dehydroisoandrosterone (III). These three compounds are 17 keto steroids and are interrelated stereochemically as shown in the following formulæ



Whereas androsterone and dehydroisoandrosterone are androgenically active 3 hydroxyaetiocholan 17 one has no such activity. In order to estimate the total 17 keto steroid content of urine it is therefore not possible to use a method of bio assay but recourse has to be made to chemical and physical tests. A colorimetric method introduced for this purpose by Zimmermann¹³⁵ and modified by Callow and Callow¹³⁶ depends on the production of a transient coloured complex with *m* dinitrobenzene in the presence of alkali. By use of suitable colour filters this test can be made reasonably specific for 17 keto steroids. More accurate determinations can be made by the polarographic analysis of a solution of the derivatives which the 17 keto steroids form with a Girard reagent. The total 17 keto steroid fraction can be further classified into 3(α) and 3(β) hydroxy 17 keto steroid fractions by treatment with digitonin which will precipitate preferentially the dehydroisoandrosterone (III) which alone of the three has the 3(β) configuration. Improved methods for the determination of the qualitative and quantitative variations in the pattern of steroid excretion in human urine in health and disease have been reported¹³⁷ and the chemical characteristics of some thirty five (α) keto steroids and seven (β) keto steroids have been described¹³⁸.

In normal males the average output of neutral 17 keto steroids has been given as 14 milligrams a day whereas the average figure for normal females is 9 milligrams. Removal of the ovaries does not appreciably affect the elimination of 17 keto steroids but in Addison's disease their elimination is markedly reduced which points to the adrenal cortex as the origin of these compounds in the female. In the male they are considered to be derived in part from the adrenal cortex and in part from the testes. Female patients with cancer of the adrenal cortex excrete excessive amounts of 17 keto steroids whereas male patients show an increased elimination of oestrogens.

EXCRETION PRODUCTS

The steroid hormones and their degradation products and derivatives are eliminated from the body mainly in the urine and there is much evidence to indicate that in many cases androgens and oestrogens are inactivated by combination with glucuronic acid or sulphuric acid. Much valuable information has been obtained from qualitative and quantitative studies on the steroid content of human urine both during pregnancy and under normal and pathological conditions. As a result of these investigations it has been possible to use the hormone content of urine (and of other body fluids) as a basis for various diagnostic tests of physiological and pathological conditions.

The excretion of oestrone commences before puberty but after puberty there is a marked increase in oestrogenic substances in the urine which vary in quantity at different stages of the menstrual cycle. During pregnancy the elimination of oestrogens increases steadily and both oestrone and oestriol are excreted in combination with glucuronic acid but after parturition the excretion decreases very rapidly and reaches normal levels within seven days. Similar changes have been observed with the oestrogen content of faeces. Appreciable quantities of oestrogens continue to be found in urine after the menopause. An improved method for the determination of oestrogens in human pregnancy urine has been described by Stevenson and Marrian¹³¹.

Progesterone seems to be rapidly metabolised in the body and much of it is excreted in the urine as pregnanediol glucuronide. Pregnanediol is normally excreted only when the corpus luteum is actively producing progesterone and the quantity increases rapidly during pregnancy. There is evidence however that the corpus luteum may not be the sole source of progesterone since Marker¹³² has shown that pregnanediol is present in appreciable quantity in the urine of bulls. A gradual fall in pregnanediol excretion during pregnancy indicates that the production of progesterone is inadequate and may result in abortion. Estimations of pregnanediol in urine which can be carried out by the method of Venning¹³³ can also be of value in the early diagnosis of pregnancy. An improved method of estimation has been reported by Huber¹³⁴.

The androgen content of urine increases gradually from early childhood both in males and females. The excretion in adults varies within wide limits and women excrete about two thirds of the quantity excreted by men. Both androsterone and dehydroandrosterone have been isolated. It is known that testosterone is rapidly metabolised in the body and part of it is excreted as androsterone.

Numerous investigations have been carried out on the excretion of hormones in menstrual irregularities and abnormal conditions during pregnancy. It has also been shown that changes in the activity of the adrenal cortex frequently result in excessive elimination of androgens and in patients with adrenal tumours there is a marked increase in the

- 40 REICHSTEIN T *Helv chim Acta* 1936 19 1107
- 50 KUIZENGA M H and CARTLAND G I *Endocrinology* 1939 24 526
- 51 REICHSTEIN T *Helv chim Acta* 1937 20 978
- 52 REICHSTEIN T *Helv chim Acta* 1936 19 29
- 53 CORYFORTH J W and ROBINSON R *Nature Lond* 1947 160 737
- 54 INHOFFEN H H and ZHILSDORFF G *Ber dtsch chem Ges* 1941 74 1911
- 55 INHOFFEN H H and ZHILSDORFF G *Ber dtsch chem Ges* 1943 76 233
- 56 Field Information Agency Technical Final Report No 996
- 57 WILDS A L and DJERASSI C *J Amer chem Soc* 1946 68 2125
- 58 DJERASSI C and SCHOLTZ C R *J Amer chem Soc* 1947 69 2404
- 59 ANGER G and MIESCHER H *Experientia* 1948 4 25 *Helv chim Acta* 1948 31 7173 1949 32 1957
- 60 ROBINSON R and WALKER J *J chem Soc* 1936 747
- 61 BACHMANN W E, KUTNER S and STEVENSON A C *J Amer chem Soc* 1942 64 974
- 62 HOFFMAN M A *et al Science* 144 100 312 *J Biol Chem* 1946 164 789
- 63 BACHMANN W E, COLE W and WILDS A L *J Amer chem Soc* 1940 62 824
- 64 JOHNSON W S, PETERSEN J W and GUTSCH C D *J Amer chem Soc* 1947 69 2947
- 65 CALLOW R H and DEANEELY R *Biochem J* 1935 29 1474
- 66 MARKER R E *J Amer chem Soc* 1935 57 1755
- 67 MARKER R E, WHITMORE F C and KATZ O *J Amer chem Soc* 1935 57 2358
- 68 DALMER O, VON WERDER F, HONIGMANN H and HEYNS K *Ber dtsch chem Ges* 1935 68 1814
- 69 RUZICKA L and WETZSTEIN A *Helv chim Acta* 1935 18 986
- 70 WALLIS H S and FERNHOLZ E *J Amer chem Soc* 1935 57 1379 1504
- 71 OFFENAUER R V *Nature Lond* 1935 135 1031
- 72 MARKER R E *et al J Amer chem Soc* 1947 64 1276
- 73 RUZICKA L and WETZSTEIN A *J Amer chem Soc* 1935 57 2011
- 74 RUZICKA L and WETZSTEIN A *Helv chim Acta* 1935 18 1264
- 75 BUTENANT A and HANSEN G *Z physikal Chem* 1935 237 89 *Ber dtsch chem Ges* 1935 68 1859
- 76 RUZICKA L, WETZSTEIN A and KACI H *Helv chim Acta* 1935 18 1478
- 77 SERINI A and NOTER H *Ber dtsch chem Ges* 1938 71 1766
- 78 MARKER R E *J Amer chem Soc* 1940 62 7543
- 79 FERNHOLZ E *Liebig in* 1933 507 128
- 80 BUTENANT A, WESTPHAL U and COBLER H *Ber dtsch chem Ges* 1934 67 1611
- 81 FERNHOLZ E *Ber dtsch chem Ges* 1934 67 1825 2026
- 82 BUTENANT A and SCHMIDT J *Ber dtsch chem Ges* 1934 67 1893 1901
- 83 BUTENANT A and WESTPHAL U *Ber dtsch chem Ges* 1934 67 2085
- 84 RUZICKA L and FUCHS W H *Helv chim Acta* 1937 20 1291
- 85 BUTENANT A and SCHMIDT J *Ber dtsch chem Ges* 1939 72 189
- 86 MEYER C, WETZSTEIN A and MIESCHER H *Helv chim Acta* 1947 30 1022
- 87 MARKER R L *et al J Amer chem Soc* 1949 62 3349 1941 63 774
- 88 MARKER R E, TSUBAMOTO T and TURNER D L *J Amer chem Soc* 1940 62 2525
- 89 MARKER R E and ROHRMANN E *J Amer chem Soc* 1939 61 3597 1940 62 518
- 90 VON LIN J, LARDON A and REICHSTEIN T *Helv chim Acta* 1944 27 821 1287
- 91 LARDON A and REICHSTEIN T *Helv chim Acta* 1944 27 713
- 92 SERINI A, LOGEMANN W and HILDEBRAND W *Ber dtsch chem Ges* 1939 72 391
- 93 STEIGER M and REICHSTEIN T *Helv chim Acta* 1937 20 1040
- 94 STEIGER M and REICHSTEIN T *Helv chim Acta* 1937 20 1164
- 95 LARDON A and REICHSTEIN T *Helv chim Acta* 1943 26 607 705 747
- 96 SARETT L H *J Biol Chem* 1946 162 601
- 97 SARETT L H *J Amer chem Soc* 1949 70 1454
- 98 FISER L F and FISER M *Natural Products Related to Ph nanthrene*

REFERENCES

- 1 SHOPPEE C W *Rep Pro Chem* 1946 43 200
- 2 BLOCH K and RITTENBERG D *J biol Chem* 1942 143 297 1942 145 625
- 3 BLOCH K, BERG B N and RITTENBERG D *J biol Chem* 1943 149 511
- 4 BLOCH K *J biol Chem* 1945 157 661
- 5 BUTENANDT A and HILDEBRANDT F *Z physiol Chem* 1931 199 243
- 6 MARRIAN G F *et al Biochem J* 1930 24 435 1931 1936 30 57 2250
- 7 GIRARD A and SANDULESCO G *Helv chim Acta* 1936 19 1095
- 8 CURTIS J M, MACCORQUODALE D W, THAYER S A and DOISY E A *J biol Chem* 1934 107 191
- 9 BEALL D and MARRIAN G F *J Soc chem Ind Lond* 1934 53 309T
- 10 CARTLAND G F, MEYER R K, MILLER L C and RUTZ M H *J biol Chem* 1935 109 213
- 11 COHEN S L and MARRIAN G F *Biochem J* 1934 28 1603
- 12 DOISY E A, VILER C D and THAYER S A *Amer J Physiol* 1929 90 379
- 13 BUTENANDT A *Naturwissenschaften* 1929 17 879
- 14 DINGEMANSE F, DE JONGH S E, KOBER S and LAQUEUR E *Dtsch med Wsch* 1930 56 301
- 15 ZONDEK B *Flm Hschr* 1930 9 2285
- 16 ZONDEK B *Nature Lond* 1934 133 709 494
- 17 DINGEMANSE E, LAQUEUR E and MÜHLBOCK O *Nature Lond* 1938 141 977
- 18 BUTENANDT A and JACOBI H *Z physiol Chem* 1933 218 104
- 19 SCHWENK E and HILDEBRANDT F *Naturwissenschaften* 1933 21 177
- 20 MACCORQUODALE D W, THAYER S A and DOISY E A *Proc Soc exp Biol NY* 1935 32 1182
- 21 MACCORQUODALE D W, THAYER S A and DOISY E A *J biol Chem* 1936 115 435
- 22 MARRIAN G F *Chem and Ind* 1930 49 237 515
- 23 MARRIAN G F and HASLEWOOD G A H *Biochem J* 1932 26 75
- 24 COHEN S L, MARRIAN G F and ODELL A D *Biochem J* 1936 30 2250
- 25 SKARZYNSKI B *Nature Lond* 1933 131 766
- 26 GIRARD A *et al CR Acad Sci Paris* 1932 194 909 1020 1932 195 981 1933 196 137 *Bull Soc Chim biol Paris* 1933 15 562
- 27 BUTENANDT A and TSCHERNING K *Z physiol Chem* 1934 229 167 185
- 28 CALLOW N H and CALLOW R K *Biochem J* 1938 32 1759 1939 33 931
- 29 BUTENANDT A, DANNENBAUM H, HANTSCH G and KUDSZUS H *Z physiol Chem* 1935 237 57
- 30 DAVID K, DINGEMANSE E, FREUD J and LAQUEUR E *Z physiol Chem* 1935 233 281
- 31 BUTENANDT A and TSCHERNING K *Z anorg Chem* 1931 44 905
- 32 RUZICKA L *et al Helv chim Acta* 1934 17 1389 1395 1407
- 33 BUTENANDT A and DANNENBAUM H *Z physiol Chem* 1934 229 197
- 34 ALLEN W M *et al Amer J Physiol* 1929 88 326 1930 92 174 1933 106 55 *J biol Chem* 1932 98 591
- 35 ALLEN W M and GOETSCH C *J biol Chem* 1936 116 653
- 36 BUTENANDT A, WESTPHAL U and HOHLWEG W *Z physiol Chem* 1934 227 84
- 37 BUTENANDT A *Flm Hschr* 1934 30 934
- 38 BUTENANDT A and WESTPHAL U *Ber dtsch chem Ges* 1934 67 1440
- 39 SLOTTA K H, RUSCHIG H and FELS F *Ber dtsch chem Ges* 1934 67 1270
- 40 ALLEN W M and WINTERSTEINER O *Science* 1934 80 190 *J biol Chem* 1934 107 321
- 41 HARTMANN M and WETSTEIN A *Helv chim Acta* 1934 17 878 1365
- 42 SWINGLE W W and PFIFFNER J J *Amer J Physiol* 1931 96 153 164 180
- 43 PFIFFNER J J *et al J biol Chem* 1934 106 625 645
- 44 CARTLAND G F and KUIZENGA M H *J biol Chem* 1936 116 57
- 45 REICHSTEIN T, LAQUEUR E, UYLDERT I E, DE FRUTERY P and SPANHOFF I W *Proc Konink Akad Wetenschap* 1936 39 1218
- 46 MASON H L, MYERS C S and KENDALL E C *J biol Chem* 1936 114 613
- 47 MASON H L, MYERS C S and KENDALL E C *J biol Chem* 1936 116 267
- 48 WINTERSTEINER O and PFIFFNER J J *J biol Chem* 1936 116 291

CHAPTER V

STANDARDISATION

DURING the past few decades there have been rapid developments in the technique of biological standardisation. Researches into the vitamins and hormones have played an important part in stimulating these improvements the more so because of the need for accurate assessment of the potency of commercial preparations. At first attempts were made to define potency in terms of animal units and there arose a welter of rat, mouse and other units for various hormones. Indeed it still seems almost inevitable that the potency of new substances will continue to be defined in such terms despite their demonstrable inadequacy. A rat or mouse unit of oestrogen for instance has been variously defined in different laboratories as the minimal amount which causes vaginal cornification in all of a group of animals, as the amount causing it in the majority of a group, or as the amount causing 50 per cent. of the animals to react. Quite apart from the vagueness of definition inherent in some of these requirements, the variability of the same stock of animals from one time to another makes any such unit unstable. Burn¹ for instance has shown how the frog unit for digitalis varied in the course of a year from 1310 to 2940 units per gramme and Emmens how the mouse unit for oestrone similarly varied from 0.64 to 1.50 international units.

These difficulties are largely resolved by the recognition of the need for standard preparations with which any other substance of similar activity may be compared. In theory so long as the mode of action of a sample of unknown potency is the same as that of the standard, an estimate of the unknown potency derived from the simultaneous comparison of the actions of the standard and unknown should be independent of the test object used and of the particular reaction chosen as a response. This situation is for the most part realised in practice although differences sometimes occur between estimates of potency when different species of animals or routes of administration are used. These are often demonstrably due to the heterogeneity of one or both of the preparations being compared, or to the presence of impurities in the unknown which affect its action differentially in various species of animal.

International Standards : The current international standard preparations designed for reference in the standardisation of hormones or hormone like substances are listed in Table VI.

The preparations are kept at various centres under conditions designed to preserve their full potency and are made available to laboratories all over the world. The distribution of standards in Great Britain is made from the National Institute for Medical Research in

- 99 MATTOX V R and KENDALL E C *J Amer chem Soc* 1948 70 882
- 99a KENDALL E C *Chem En n, News* 1950 28 2074
- 100 SOLMSEN U V *Chem Reviews* 1945 37 481
- 101 COOK J W DODDS E C HEWETT C L and LAWSON W *Proc roy Soc Series B* 1934 114 272
- 102 DODDS E C and LAWSON W *Proc roy Soc Series B* 1938 125 222
- 103 DODDS E C GOLBERG L LAWSON W and ROBINSON R *Proc roy Soc Series B* 1939 127 140
- 104 CAMPBELL N R DODDS E C and LAWSON W *Proc roy Soc Series B* 1940 128 253
- 105 KHARASCH M S and KLEIMAN M *J Amer chem Soc* 1943 65 11
- 106 BOOTS PURE DRUG CO LTD and SHORT W F British Patent 523370
- 107 DOCKEN A M and SPIELMAN M A *J Amer chem Soc* 1940 62 2163
- 108 BERNSTEIN S and WALLIS E S *J Amer chem Soc* 1940 62 2871
- 109 BOOTS PURE DRUG CO LTD HOBDAI G I and SHORT W F British Patents 566723 566724
- 110 HOBDAI G I and SHORT W F *J chem Soc* 1943 609
- 111 JONES E R H *Rep Progr Chem* 1943 40 137
- 112 DODDS E C FITZGERALD M E H and LAWSON W *Nature Lond* 1937 140 772
- 113 ROBSON J M and SCHONBERG A *Nature Lond* 1937 140 196
- 114 ROBSON J M SCHONBERG A and FAHIM H A *Nature Lond* 1938 142 292
- 115 DAVIES J S H and IMPERIAL CHEMICAL INDUSTRIES LTD British Patent 549200
- 116 BASFORD F R and IMPERIAL CHEMICAL INDUSTRIES LTD British Patent 559374
- 117 ROBSON J M SCHONBERG A and TADROS W *Nature Lond* 1942 150 27
- 118 CARTER P R and HEY D H *J chem Soc* 1948 150
- 119 RUZICKA L GOLDBERG M W and ROSENBERG H R *Helv chim Acta* 1935 18 1487
- 120 RUZICKA L *et al Helv chim Acta* 1937 20 1280 1938 21 371
- 121 INHOFFEN H H LOGEMANN W HOHLWEG W and SERINI A *Ber dtsch chem Ges* 1938 71 1024
- 122 INHOFFEN H H and HOHLWEG W *Klin Wschr* 1939 18 77
- 123 INHOFFEN H H and HOHLWEG W *Naturwissenschaften* 1939 26 96
- 124 MARRIAN G F and HASLEWOOD G A D *J Soc chem Ind Lond* 1932 51 277T
- 125 MACCORQUODALE D W LEVIN L THAYER S A and DOISY E A *J biol Chem* 1933 101 753
- 126 HEER J and MIESCHER K *Helv chim Acta* 1945 28 156
- 127 HEER J BILLETER J R and MIESCHER K *Helv chim Acta* 1945 28 991
- 128 HEER J BILLETER J R and MIESCHER K *Helv chim Acta* 1945 28 1342
- 129 ANNER G and MIESCHER K *Helv chim Acta* 1946 29 580
- 130 SHOPPEE C W *Rep Progr Chem* 1947 44 190
- 131 STEVENSON M F and MARRIAN G F *Biochem J* 1947 41 507
- 132 MARKER R L WITTLE E L and LAWSON E J *J Amer chem Soc* 1938 60 2931
- 133 VENNING E H *J biol Chem* 1937 119 473
- 134 HUBER D *Biochem J* 1947 41 609
- 135 ZIMMERMANN W *Z physiol Chem* 1935 233 257
- 136 CALLOW N H and CALLOW R K *Biochem J* 1938 32 1759 1939 33 559 931
- 137 DOBRINER K LIEBERMAN S and RHOADS C P *J biol Chem* 1948 172 241
- 138 LIEBERMAN S DOBRINER K HILL B R FISHER L F and RHOADS C P *J biol Chem* 1948 172 263

commercial scale in a similar state of purity. Once the production of the hormones as pure chemical substances is achieved there is no object in continuing to use biological methods of estimation nor when it is achievable should manufacturers be content to produce impure products.

BIOLOGICAL ASSAY

A satisfactory biological assay must fulfil certain specific requirements the relaxation of which should be permissible only in very special circumstances. The minimal requirements are

- (i) A standard reference preparation must be used simultaneously with the preparation under test
- (ii) The assay must provide a valid unbiased estimate of the potency of the preparation under test and of the limits of error of this estimate at any required level of probability
- (iii) The assay must provide evidence that the actions of the preparation under test and of the standard preparation do not differ

The first requirement guarantees that the comparison of standard and unknown shall be independent of secular variation in response.

The second requirement concerns both the conduct of the test and its statistical evaluation. It implies in general that the line relating the response to the dose or the logarithm of the dose (log dose) should be straight and that its slope should be determined on each occasion that an assay is performed; that the variance of the response should in general be independent of the level of the response in assays based on graded responses; and that the statistical methods by which the potency and its limits of error are determined should be mathematically sound. It also implies that the animals or other test objects used in the assay are assigned although perhaps within certain permissible restrictions strictly at random to doses and substances.

The third requirement is satisfied only when at least two different dosage groups are used for both the standard and the unknown. When this is done the parallel nature of the two dose response lines can be examined and thus the similarity of action of the two substances is checked. More than two dosage groups for each substance should be used unless there is already sufficient evidence that the dose response relationship is linear over the range encountered in the assay.

Further characteristics of a satisfactory test method have been outlined by Gaddum³ and by Bliss⁴. These may be summarised as follows.

- (iv) The most accurate test method will be that for which the quantity s/b is minimal where s is the standard deviation of an individual result and b is the slope of the dose response line.
- (v) The living material receiving each dose of the standard and unknown must be as uniformly distributed among such dosage groups as is possible. Potential sources of variation such as differences in response between litter mates, sexes or strains of

London In order to conserve these preparations it is intended that laboratories should use them for the careful standardisation of their own local reference standards so that estimates of potency made by comparison with these local standards shall be referable with little error to the international standard preparation

TABLE VI
INTERNATIONAL STANDARD HORMONE PREPARATIONS

<i>Preparation</i>	<i>Date adopted</i>	<i>Unit (milligrams)</i>
Insulin (crude dry insulin hydrochloride)	1925	0.125
Insulin (pure crystalline insulin)	1935	0.0455
Hypophyseal (posterior lobe) powder	1925	0.5
Oestrone	1932	0.0001
Oestradiol monobenzoate	1935	0.0001
Androsterone	1935	0.1
Progesterone (corpus luteum hormone)	1935	1.0
Chorionic gonadotrophin	1938	0.1
Serum gonadotrophin	1938	0.25
Prolactin (galactin or mammotrophin)	1939	0.1

For each standard there is also an international unit. This unit is the activity of a specified weight of each standard different for different standards and was usually defined for convenience with reference to the approximate quantity of standard required to elicit a response in a particular biological test which was popular at the time of determining the unit. Since vaginal cornification in the mouse was and still is a method most frequently employed in the estimation of oestrogens the international unit for oestrone became the very small quantity 0.0001 milligram of the standard while the use of progestational changes in the rabbit uterus in the assay of progesterone led to the adoption of 1.0 milligram of this substance as a unit. There is thus no general relationship between the weight of an international unit and the dosage required in medicine. When an international standard exists however the potency of a preparation must be stated in terms of the number of units in one millilitre or gramme to which its action is equivalent. The introduction of these standards has therefore meant that the potency of a preparation made anywhere can be accurately measured and compared with that of another without their physical proximity, and that the dosages of various drugs used in medicine can be so controlled that the safety of the patient and the reputation of the manufacturer are guarded alike.

It should be noted that such standards are needed only when it is impossible or inconvenient to characterise a drug chemically. Some existing standards in particular those relating to the sex hormones are rapidly falling into disuse since the standard preparations themselves are pure crystalline substances which can now be produced on a

The variance of a probit is not constant for different percentages and the dose response line has therefore to be fitted by using weights which are inversely proportional to the theoretical variance. The line is fitted by a series of successive approximations and although only one or two cycles of calculation may be needed the arithmetic tends to become irksome even when equal numbers of test objects have been placed at each dosage level. There are various approximate methods for estimating relative potency which often give a sufficiently good answer but none is adequate for the assessment of limits of error.

Choosing a Test method Factors such as cost, time and labour requirements will often strongly influence the choice of a particular test method for assaying preparations and it is rarely that a method combines optimal precision with desirable levels of these other factors. High sensitivity is sometimes important as when insufficient quantities would be available for a more accurate but less sensitive method but it is not otherwise necessary in standardisation. Specificity is of course of the highest importance. A response which can be measured quantitatively is usually to be preferred since quantal responses are more troublesome statistically and usually give less accurate estimates for the same number of observations.

A simple measure of response such as the weight of an organ is more likely to give a linear log dose response relationship than are less natural measures such as percentage increase in size or weight. It is usually pointless to express the response in terms of some other variable such as body weight until the proper relationship has been established. Thus although it might lead to a more accurate estimation of potency in a particular test if the weight of an organ be expressed as a percentage of the carcass weight instead of using the crude weight neither method is likely to give as accurate a result as the use of a correction factor derived by covariance analysis. Similarly it seems to be widely assumed that when both initial and final measurements of the test object are possible in assays it is more accurate to use some such function as the difference between them as a criterion of response. This may or may not be true frequently it is not. Instead covariance analysis will enable a critical appraisal of the situation to be made.

Computation The literature abounds with approximate and sometimes completely incorrect methods of computing relative potency and its errors. Most of the approximate methods give no better an answer than can be guessed by anyone accustomed to dealing with assay data none of them gives a more accurate estimate of potency than a simple graphical fitting by a careful worker. Moreover in actual practice some of them are more time consuming than the exact computation of potency from a balanced assay although it must be admitted that the computing of fiducial limits of error usually takes somewhat

animal must be so allocated to dosage groups that their influence can be isolated and examined in the subsequent statistical analysis. Doses should not in general be adjusted for such factors as body weight by assuming some arbitrary and perhaps incorrect relationship but from the internal evidence of the test or group of tests of a like nature. The statistical method appropriate to the making of such adjustments is covariance analysis; an excellent example is given by Bliss and Marks⁵ using the assay of insulin to exemplify the procedure.

In hormone assays it is usually found that the logarithm of the dose is linearly related to the response. Under these conditions there is rarely any point in failing to distribute test objects equally among the same number of dosage groups of both standard and unknown and to arrange doses so that successive dosage groups for each substance are spaced at the same equal logarithmic intervals. Thus if sixty animals are available for a test of a particular substance against the standard in an assay in which it is deemed advisable to use three doses of each preparation, each dose should be given to ten animals and doses should be in geometrical progression, say 1, 2, 4 units of standard and 2, 4, 8 milligrams of unknown, not in arithmetical progression, say 1, 2, 3 units and 2, 4, 6 milligrams. When this scheme is followed computation is reduced to a minimum and the amount of information gained from the test is maximal.

When several unknowns are simultaneously compared with the standard, the rule requiring equal distribution of test objects between substances may be modified, since the maximal amount of information about the potency of each unknown will on an average be obtained from an arrangement by which each dose of the standard is given to \sqrt{N} times as many test objects as is each dose of each unknown, where N is the number of unknowns. It is not worth worrying about this in practice unless many unknowns are used at a time, since the statistical computations are more laborious and the gain in information is not very great when N is small.

Quantal Responses. When all that is recorded about a group of test objects is whether or not each of its members exhibits some characteristic effect, such as death or vaginal cornification, the response is called quantal, and special methods are needed in dealing with the data. If the percentage response is plotted against either the dose or log dose, the line relating the two is usually *S* shaped (sigmoid). To obtain a straight log dose response line, it is necessary to convert the percentage of response to probits, which are calculated on the assumption that the logarithms of the individual effective doses of the preparations under test are normally distributed.^{6, 7} Tables of probits corresponding to percentages of response from 0.1 to 99.9 are given by Bliss, and in more detail by Fisher and Yates.⁸

Limits of Error It is usual to accept as the limits of error of an assay a range which is estimated to include the true potency in either 95 or 99 per cent of cases. When large numbers of test objects are used, this range is $M \pm 1.96 s_M$ for the 95 per cent limits and $M \pm 2.58 s_M$ for the 99 per cent limits. With numbers below a hundred, however, and particularly with small total numbers like twenty or thirty animals in a test, the distribution of t should be used as given in any modern textbook of statistics, and the range $M \pm t s_M$ calculated. Thus with a total of forty animals in a test, distributed between four groups, t would be based on 36 degrees of freedom (or even less, if such a factor as litter or strain difference is segregated in the test); the 95 per cent range would be $M \pm 2.03 s_M$ and the 99 per cent range $M \pm 2.72 s_M$. In quantal assays, however, the value of t remains 1.96 and 2.58 for the two probability levels, whatever the number of animals employed.

The error of any particular single assay cannot be predicted, except within wide limits, but the average error or the minimal expected error can be used for comparing the precision of different methods. In the following pages it is imagined that a standard assay, using a graded response, is conducted with forty animals, which will usually be distributed between four dosage groups, two for each preparation. When possible, the estimated minimal error for $P = 0.95$ is calculated for each method of assay ($P = 0.95$ implies that the chance that the true value lies outside the range given is only 1 in 20). This error may be substantially exceeded in practice, in particular if the mean responses to standard and unknown differ widely, and if the value of the slope is not accurately known. The minimal value expected for s_M in such a standard assay is $\frac{s^2}{10b^2}$ in a log dose response test, supposing b to be

determined with sufficient precision for the separate calculation of fiducial limits of error to be unnecessary. This would often mean that an average value of b from previous assays must be available and be consistent with the new estimate of b provided by the assay being examined.

The use of litter mates decreases error very considerably in all tests in which it has been investigated. This decrease is of such magnitude that it is always worth the trouble of using litter mates when possible, since the average test employing them has two to four times the accuracy (i.e. one half to one quarter of the variance) of a corresponding test with animals selected at random. When possible, it is similarly desirable to use cross over techniques, in which the same animals are used at different times on the standard and unknown preparations in a test which is so balanced that overall differences in sensitivity between animals and differences due to secular variation are eliminated. The elimination of differences between animals may reduce the error mean square to as little as one sixth of the value found without such a technique. The errors quoted below are not calculated on a litter mate or cross over basis, except where specifically stated.

longer. The use of balanced test methods of the type briefly described above permits factorial analysis of the assay, which is completed in a short time.

Approximate methods are of most help when quantal responses are involved. Here exact calculation tends to be more prolonged but the approximation is best confined to simple graphical estimation of potency. No simple and sufficiently accurate approximate method is available for the estimation of limits of error, however, and as these should be determined as a routine except in trivial circumstances the incidental determination of potency which their computation involves makes the approximate determination of potency superfluous. The computational procedures for all types of assay discussed here are described by Emmens⁹. These computations consist in essence of the determination of the following quantities when one unknown is compared with a standard:

- (i) The mean log dose of each preparation \bar{X} and \bar{Y} for standard and unknown respectively
- (ii) The mean response to each preparation, \bar{I}_s and \bar{I}_u
- (iii) The common slope b
- (iv) The standard deviation of a single observation s
- (v) The standard error of the slope s_b

The logarithm of the ratio of the potency of the unknown to that of the standard M is given by

$$M = (\bar{Y} - \bar{X}) - \frac{(\bar{I}_u - \bar{I}_s)}{b}$$

where \bar{X} and \bar{Y} are measured in the same units such as milligrams. The error of M is inherent only in the second term on the right hand side since there should be no significant error in administering doses. If b is estimated with relatively high accuracy which in practice means that b/s_b is greater than about 8 then s_M the standard error of M is such that

$$s_M^2 = \frac{s^2}{b^2} \left(\frac{1}{n} + \frac{1}{n_u} \right) + \frac{(\bar{I}_u - \bar{I}_s)^2}{b^4}$$

where n and n_u are the numbers of observations on the standard and unknown respectively. It will be seen that a minimal value for s_M occurs when $\bar{I}_u = \bar{I}_s$ and that the greater the difference in mean response the lower the accuracy of the assay.

If b/s_b be less than about 8 this formula for s_M is not sufficiently accurate for the determination of limits of error and the so-called fiducial limits of error should be calculated by more exact means. These are discussed by Irwin¹⁰. The estimation of M however is not affected.

laboratory and a dose response curve is given perhaps with standard errors of groups often for one preparation only. Sometimes no actual assays have been conducted by a particular method and the reader is unable to decide whether even in the author's hands it gives reliable results. In the absence of sound information as to their reliability it is therefore impossible to give more than passing mention or perhaps to do full justice to many suggested procedures.

ANTERIOR LOBE OF THE PITUITARY

Thyrotrophic Hormone The setting up of an international standard for the thyrotrophic hormone was decided on in 1938¹¹ but has not yet been completed. There is therefore no standard preparation or international unit in terms of which thyrotrophic extracts of the anterior lobe can be calibrated.

There is only one method which can be particularly recommended for the assay of the thyrotrophic hormone that utilising the increase in weight of the thyroid gland of the guinea pig as described by Rowlands and Parkes¹². In guinea pigs injection of the thyrotrophic hormone causes hyperplasia of the thyroid gland (it does not do so in the rat which is therefore unsuitable for this type of test) and the weight of the stimulated gland is proportional to the logarithm of the dose over a wide range. The method is described by Burn¹.

Rowlands and Parkes¹² used female guinea pigs approximately 200 grammes in weight but there seems to be no objection to using males instead. The weight of the unstimulated gland appears to be linearly related to body weight and thus the use of animals of a relatively wide weight range might prove as accurate as long as the body weight is used as a concomitant variable in statistical analysis. Subcutaneous injections are given for five days and the animals are killed on the sixth day. The glands are fixed in Bouin's fluid overnight and weighed at the alcohol (70 per cent) stage in upgrazing. It is necessary to know the mean unstimulated weight of glands in the stock being used to ensure that a response is in fact being produced by the lowest doses employed.

There is insufficient evidence upon which to base reliable estimates of the error of this test but it would appear that the minimal limits of error ($P = 0.95$) to be expected when two groups each of ten guinea pigs are used with each substance (forty animals all told) are about 77 to 129 per cent of the estimated potency.

The thyroid gland of the chick also responds to the thyrotrophic hormone by an increase in weight and the same general test method may be employed using the chick thyroid as the test object as described by Bergman and Turner¹³. The authors state that this method is not so good as the guinea pig test but that it is more convenient when chicks are easily obtained. However from the standard errors and slopes reported by Bergman and Turner it looks as if the chick test using day old White Leghorns is more accurate in their hands than

In quantal assays the standard test is increased to eighty animals because no worthwhile degree of precision can usually be expected with fewer test objects and the chance of obtaining 0 or 100 per cent of responses with only ten animals per group is too high for practical safety.

In such assays it is also assumed that the average weight factor⁴ is 0.5. This gives a slightly lower weight to a group than it might often have in practice and slightly wider limits of error than might be expected if all groups gave responses of between 20 and 80 per cent.

METHODS OF ASSAYING RELATIVE POTENCY

The rest of this chapter is a survey of methods for standardising hormone preparations. These cannot be given in complete detail and reference should be made to the original papers if it is desired to carry out an assay by a particular method or to descriptions given by Burn¹ and the British Pharmacopœia. If Burn or the British Pharmacopœia has described a method in full reference is made to the fact. It should be remembered however that many good assay methods have not been described by their authors in the light of modern statistics and that frequently the reader is advised to use a single group of test-objects and to refer to a standard curve given by the author of the method or to a similar curve which has been determined in his own laboratory or to conduct his assay by some other equally hazardous procedure. Such recommendations represent a phase in the development of the subject which should by this time be disappearing. It will therefore bear stressing again that satisfactory assays by whatever biological method the response is obtained supply internal evidence of their own reliability and that the primary basis of this is the simultaneous comparison of standard and unknown preparations with at least two dosage groups for each.

In selecting test methods for particular recommendation it has also to be borne in mind that many methods recently developed for hormone assay have been devised primarily with the object of detecting small quantities in extracts of urine or tissues. Thus sensitivity has been a first requirement and much ingenious work has been put into converting such sensitive tests of the presence of a hormone into satisfactory quantitative methods of assay. Frequently however these methods are not as accurate as older techniques which use larger amounts of material and are therefore not to be preferred for the purposes of standardisation. In general histological methods are not recommended because they are troublesome and usually incapable of satisfactory quantitative expression.

There is a further difficulty which faces the reviewer of methods of assay. Many suggested procedures look very promising, some of them put forward as improvements in existing methods, others quite new. Too frequently however a method has been studied only in one

laboratory, and a dose response curve is given perhaps with standard errors of groups often for one preparation only. Sometimes no actual assays have been conducted by a particular method and the reader is unable to decide whether even in the author's hands it gives reliable results. In the absence of sound information as to their reliability it is therefore impossible to give more than passing mention or perhaps to do full justice to many suggested procedures.

ANTERIOR LOBE OF THE PITUITARY

Thyrotrophic Hormone The setting up of an international standard for the thyrotrophic hormone was decided on in 1938¹¹ but has not yet been completed. There is therefore no standard preparation or international unit in terms of which thyrotrophic extracts of the anterior lobe can be calibrated.

There is only one method which can be particularly recommended for the assay of the thyrotrophic hormone that utilising the increase in weight of the thyroid gland of the guinea pig as described by Rowlands and Parkes¹². In guinea pigs injection of the thyrotrophic hormone causes hyperplasia of the thyroid gland (it does not do so in the rat which is therefore unsuitable for this type of test) and the weight of the stimulated gland is proportional to the logarithm of the dose over a wide range. The method is described by Burn¹.

Rowlands and Parkes¹² used female guinea pigs approximately 200 grammes in weight, but there seems to be no objection to using males instead. The weight of the unstimulated gland appears to be linearly related to body weight and thus the use of animals of a relatively wide weight range might prove as accurate as long as the body weight is used as a concomitant variable in statistical analysis. Subcutaneous injections are given for five days and the animals are killed on the sixth day. The glands are fixed in Bouin's fluid overnight and weighed at the alcohol (70 per cent) stage in upgrading. It is necessary to know the mean unstimulated weight of glands in the stock being used to ensure that a response is in fact being produced by the lowest doses employed.

There is insufficient evidence upon which to base reliable estimates of the error of this test but it would appear that the minimal limits of error ($P = 0.95$) to be expected when two groups each of ten guinea pigs are used with each substance (forty animals all told) are about 77 to 129 per cent of the estimated potency.

The thyroid gland of the chick also responds to the thyrotrophic hormone by an increase in weight and the same general test method may be employed using the chick thyroid as the test-object as described by Bergman and Turner¹³. The authors state that this method is not so good as the guinea pig test but that it is more convenient when chicks are easily obtained. However from the standard errors and slopes reported by Bergman and Turner it looks as if the chick test using day old White Leghorns is more accurate in their hands than

the guinea pig test. They also report a linear dose response relationship not a linear log dose response relationship. Males are preferred to females because of a steeper slope.

Lactogenic Hormone The international unit of the lactogenic hormone (prolactin) is the specific activity of 0.1 milligram of the international standard preparation.

It is generally agreed that the growth of the crop glands of male or female pigeons provides a valid estimate of the lactogenic activity of preparations of the anterior lobe: this was the only test recognised by the Third Conference on the Standardisation of Hormones held in Geneva in 1938¹¹ as giving sufficiently quantitative results for the comparison of preparations.

The original method of Riddle, Bates and Dykshorn¹⁴ was to inject intramuscularly once daily for four days and to kill at ninety six hours. The crop gland weight in milligrams was then multiplied by 150 and divided by the body weight and was found to be linearly related to

$\log \left(\frac{\text{dose} \times 150}{\text{body weight}} \right)$ It is not very clear why this was done and later

workers have successfully used simpler relationships. Body weight has an influence on response and should be corrected for by covariance analysis. When six daily injections were given and the birds were killed on the seventh day the mean slope relating crude crop gland weight in grammes to the logarithm of the dose for nine preparations was approximately 7.1¹⁵ and the variance of the crop gland weight at the 4 gramme level of response was about 1.92. The minimal expected limits of error in a test using forty birds distributed equally between test and standard preparations is thus of the order of 75 to 133 per cent ($P = 0.95$) and should be reduced if proper allowance be made for the correlation between body weight and response.

Various modifications of the pigeon test involving the local application of the preparations under test have been tried as described for instance by Reece and Turner¹⁶, Meites, Bergman and Turner¹⁷ and Hall¹⁸. The general conclusion seems to be that although this type of test is much more sensitive it is not very consistent. Hall however injects small doses locally over the crop sac either subcutaneously or intradermally using the left and right sides of the same bird for the standard and unknown and concludes that this modification gives reliable results. Such a method could not readily be adapted for assaying preparations however unless a technique using the design known as symmetrical pairs were used as described by Bliss and Rose¹⁹. When the figures submitted by the laboratories taking part in the examination of the contributions offered towards the international standard preparation were examined by Emmens²⁰ it was clear that the intradermal tests there reported were not as reliable as the more orthodox technique

Summaries of a variety of methods used in the assay of the lactogenic hormone are given in the Appendix to Emmens⁹

Growth Hormone · No international standard exists for the estimation of the activity of growth promoting preparations of the anterior lobe of the pituitary gland

The estimation of growth promoting activity is usually made with rats either normal or hypophysectomised and the criterion of response may be an increase in body weight or various skeletal changes. Marx, Simpson and Evans²¹ have made a careful comparison of the use of plateaued normal female rats as against hypophysectomised female rats for the growth test. A plateaued rat is one which has reached a body weight of 220 to 280 grammes and is growing very slowly. A rapid burst of growth may however be stimulated by the injection of the growth hormone. Hypophysectomised rats are more sensitive to the growth hormone than plateaued animals but are more difficult to prepare and handle (see Burn¹).

A reasonably steep slope for the dose response curve is attained only after about fifteen days of injections which was adopted as a test period. Marx *et al.*²¹ present their data in a form which makes statistical comparisons difficult but by reading back from their figures and tables it has been possible to estimate roughly the relative accuracy of the two methods. The approximate slope of the line for plateaued rats is 27 and for hypophysectomised rats 18 when the weight increase in grammes is plotted against log dose. The variances are about 47 and 43 respectively. The expected minimal limits of error ($P = 0.95$) if forty rats are used in a balanced test are therefore 69 to 145 per cent for plateaued animals and 60 to 170 per cent for hypophysectomised animals. The authors say that the accuracy is about the same for both types of test or even somewhat higher for normal rats. They have tested many preparations and find a remarkably consistent value for the slope for plateaued rats which may therefore be recommended as suitable test objects. Griffiths and Young² have described a different type of plateaued rat test. Their rats received implantations of tablets of stilboestrol a procedure which arrests growth and simulates hypophysectomy in many ways. Such animals also respond to the action of the growth hormone but no critical evaluation of the accuracy of assays by this method is possible at present nor is it recommended by the authors as being particularly suitable.

Evans, Simpson, Marx and Kubrick²² have also elaborated a test using hypophysectomised rats in which the criterion of response is the width of uncalcified epiphyseal cartilage in the tibia. This test is stated to be three times as sensitive as the body weight method on a daily dose basis and since it takes only four days instead of fifteen it requires only about one tenth of the total dose. From the figures in the text this method would appear to be quite accurate and the log dose response

line seems excellent giving a linear relationship over a range of from 5 to 200 micrograms of a local standard preparation. The mean error of the test is stated to be 0.42 micrometer divisions but it is not clear whether this applies to means of groups or to individual observations. Since the slope relating width of cartilage in scale divisions to log dose is 4.3 the minimal expected error of an assay is ($P = 0.95$) 86 to 116 per cent if twenty rats are used on the standard and twenty on the unknown and if 0.42 is in fact the standard deviation for individuals. These limits seem very narrow for such a test, and it would appear that the error cited is that of means but since no details of the numbers involved are given it is not possible to estimate the probable errors of the test.

Adrenocorticotrophic Hormone There is no international standard for the adrenocorticotrophic hormone. Work on the standardisation of the hormone has been based on increases in adrenal weight in rats by Moon^{24, 25} or in chicks by Bates, Riddle and Miller²⁶. No details of errors are given by Moon and the value of his tests cannot therefore be estimated. Bates *et al.* however present sufficient data for a rough estimate of the errors of the test to be made. They injected two-day old White Leghorn cockerels subcutaneously three times a day for five days. The dose response relationship was found to be linear but from the look of the figures the log dose response relationship also is probably linear over a wide enough range for assay purposes. However, it is often more accurate to use a linear dose response relationship if this is possible. The best fitting line for one series of chicks was adrenal weight in milligrams = $0.267 X + 10.1$ where X was the dose of a particular preparation in milligrams. The standard error of the mean of groups of ten chicks was said to be usually less than 0.7 milligram at low levels and rarely over 1.0 milligram at high levels of response. If we take 0.7 as the average standard error to be expected using the dose response line above and distribute forty animals equally among five groups in a common zero.5 point assay as described by Finney²⁷ the expected limits of error ($P = 0.95$) will be approximately 80 to 120 per cent it being assumed that as wide a dosage range as is covered by the dose response line (up to 40 milligrams) is used. In this type of assay a fifth group on zero dose is included in the calculations and the two dose response lines for standard and unknown are constrained to intersect at zero dose.

The adrenal weight test using hypophysectomised forty day male rats has been investigated by Simpson, Evans and Li.⁸ It was treated by these authors as a test of the maintenance of adrenal weight in comparison with normal forty day controls but is adaptable as an assay in which the actual adrenal weight is used as the response. The log dose response relationship appears linear when fifteen daily injections are given with a slope of about 9.5 when adrenal weight in

milligrams is plotted against the logarithm of the dose in milligrams. The average variance was about 13 hence the minimal limits of error ($P = 0.95$) would appear to be in the neighbourhood of 57 to 174 per cent with the usual forty animal test used here as a basis for comparison.

Sayers, Sayers, Tsan Ying and Long²⁹ have described a possible assay method using the fall in adrenal ascorbic acid and adrenal cholesterol which follows the administration of the adrenocorticotrophic hormone to both rat and guinea pig. A similar study has been made by Reiss and Halkerston³⁰.

Gonadotrophins Methods for the assay of gonadotrophins of the anterior lobe are essentially similar to those described below for serum gonadotrophin and chorionic gonadotrophin. As the content of follicle stimulating hormone and luteinising hormone varies from species to species it is usually necessary to use hypophysectomised test animals for differentiating between the two in a mixture. No quantitative data are available from which to recommend an assay procedure under such circumstances nor does it seem to be in demand for the examination of commercial preparations. Thus any assay of an anterior lobe preparation will as matters stand at present necessarily be approximate and the answer will vary according to the conditions of the test. There is no international standard pituitary gonadotrophin.

CHORIONIC GONADOTROPHIN

The international unit of chorionic gonadotrophin is the specific gonadotrophic activity of 0.1 milligram of the international standard preparation and it is to be used only for recording the activity of preparations of human pregnancy urine.

There is a great variety of tests for gonadotrophic activity. An opportunity to compare the efficiency of the more accurate methods occurred when the international standard was set up³¹ because the various contributions offered towards the standard were assayed by thirty-two different laboratories and many laboratories used several test methods. However partly owing to the method of distribution of the contributions they were not all compared with each other by simultaneous assay and the errors of the assays examined are therefore not typical of an optimal procedure. Nevertheless little doubt remained that tests employing vaginal cornification in the immature female rat or mouse, ovarian weight in the rat and the formation of corpora lutea in the rat or mouse were the most accurate probably in the order given. The vaginal cornification test and the ovarian weight test did not differ significantly in the magnitude of their error with the proviso that it was not always clear exactly how many animals had been employed in making the comparison in each case. The numbers employed were however approximately equal.

It has been decided to recommend the ovarian weight test using the immature female rat. The reasons for this recommendation are

- (i) It is more specific and does not demand control tests on spayed animals
- (ii) It is a method which can also be recommended for the assay of serum gonadotrophin
- (iii) When litter mates are used it is more accurate than any other test yet described

This method originally elaborated by Deanesly³ is given in detail in the British Pharmacopœia. It employs 40 to 80 grammes rats which are injected daily for five days and killed on the sixth day. The ovaries are dissected, fixed in Bouin's fluid and weighed at the alcohol (70 per cent) stage in upgridding. Ovarian weight is linearly related to log dose and a maximal weight of about 40 milligrams is attained with chorionic gonadotrophin. If litter mates are available these should be distributed between all doses of standard and unknown. The qualitative identity of action of the standard and unknown may be checked by histological examination of the ovaries: the characteristic action of chorionic gonadotrophin is luteinisation. The minimal limits of error ($P = 0.95$) to be expected when litter mates are used and there are forty rats in the test are 84 to 119 per cent. When litter mates are not used, the limits ($P = 0.95$) are 70 to 140 per cent.

The vaginal cornification method has the advantage that no dissection is required and that the animals need not be killed. Its disadvantages are the need for control tests to ensure the absence of oestrogenic material in the preparation under test and the fact that in the hands of some investigators it has often proved quite unreliable. This it should be noted was not so in the many laboratories taking part in the assays mentioned above. In these laboratories the slope of the probit log dose lines varied between 5.0 and 8.5. With the lower value of the slope, the limits of error ($P = 0.95$) of a test with twenty animals to a group would be at a minimum 75 to 134 per cent. With the higher value the limits would be 84 to 119 per cent.

Heard and Winton²² used adult female rats which had been placed on a diet deficient in vitamins of the B group and had ceased to exhibit œstrus cycles. The slope for tests with these animals was 8.7 so that the expected minimal limits of error for an assay with eighty such animals would also be about 84 to 119 per cent.

It may be noted that the range of slopes reported for these vaginal cornification tests is on the whole higher than those found when vaginal cornification is used as the criterion of response in the assay of œstrogens (see below). This is quite feasible and would apparently imply that equal increments in log dose elicit more than equal increments in log œstrogen production in the ovaries of the test animals.

Tests involving uterine weight, the weights of male organs or vaginal opening in rodents, ovulation in the rabbit and histological changes of

various types in these species are not in general to be recommended. They are either inaccurate or inconvenient and often both. Various test methods are however summarised in the Appendix to Emmens³¹

SERUM GONADOTROPHIN

The international unit of serum gonadotrophin is the specific gonadotrophic activity of 0.25 milligram of the international standard preparation and it is to be used only for recording the activity of preparations of pregnant mares' serum.

The same tests may be used for comparing serum gonadotrophins as for comparing chorionic gonadotrophins. When the assays of contributions towards the international standard preparation were examined³⁴ it was found that the method using ovarian weight in the rat was significantly more accurate than the general run of other tests. There were insufficient data from which to judge the accuracy of the vaginal cornification test separately. There was however no evidence that the various less accurate tests were giving biased estimates and it was concluded that independent of their accuracy they were measuring the same thing. This means that since preparations of the serum of pregnant mares are known to exert both a follicle stimulating and a luteinising activity (although predominantly the former) the various contributions to the standard must be supposed to have contained the two principles in an approximately constant ratio if in fact there be two separate hormones.

Under the stimulus of serum gonadotrophin the ovaries of the immature female rat may reach a weight of about 220 milligrams. It is not however advisable to use the upper half of the dose response line because the variance is high and there is danger of rupturing large follicles when dissecting or handling the ovaries. It is not necessary to give daily injections since a single subcutaneous dose of serum gonadotrophin given six days prior to killing the animals is effective. The qualitative identity of the standard and unknown may be checked by histological examination of the ovaries: the characteristic action is stimulation of follicular growth. In a forty rat test with litter mates the minimal limits of error ($P = 0.95$) to be expected are 93 to 108 per cent. When litter mates are not used these limits of error are 84 to 119 per cent.

POSTERIOR LOBE OF THE PITUITARY

The international unit of extracts of the posterior lobe is the specific activity (oxytocic, antidiuretic or pressor) contained in 0.5 milligram of the standard preparation when extracted by the prescribed method which is given in the British Pharmacopoeia.

Oxytocic Activity The usual method of assay employs the uterus of the virgin guinea pig and is described in the British Pharmacopoeia.

It depends on the contractions of 1 uterine horn suspended in a bath containing Tyrode solution in response to the addition of an extract of the posterior lobe. As it stands this assay is more an art than a science and cannot be assigned definite limits of error. It would appear that so long as an experienced worker is satisfied with the way in which his particular uterine preparation is responding and so long as he makes valid comparisons of the type $x \ y \ x$ where x is 1 dose of the standard and y a dose of the unknown given in that temporal order the error of the test ($P = 0.95$) is usually about ± 15 per cent.³³

Hamburger³⁴ has described a method in which the average height of the contractions produced by several determinations at each of several dose levels of both standard and unknown is measured and the test is put on a more self contained and quantitative basis. Hamburger measured twenty to thirty contractions with each uterus ten to fifteen on each preparation and constructed dose response curves for each. These curves were either approximately linear or contained a sufficiently linear segment to allow of the application of the usual statistical methods for comparing two substances although in actual practice Hamburger made graphical comparisons. Repeated determinations of the potency of three unknown dilutions of an extract of the posterior lobe were made by this method each with 1 different uterus. The true potencies were 0.25, 0.80 and 0.35 and the means of seven or eight tests were 0.24, 0.77 and 0.31 respectively. The percentage standard deviation of these three groups of tests was 13.5, 10.6 and 10.0. The limits of error ($P = 0.95$) of a single test with one uterus are therefore approximately ± 20 per cent to ± 30 per cent but only when the technique of Hamburger is followed. The usual technique would be expected to have a greater error than this because it employs less than twenty to thirty contractions in the total comparison and it is rather surprising that Gaddum⁶ found a smaller error. Bachinsky *et al.*³⁷ measured the height of responses in a test in which each uterine horn was cut into four segments the eight pieces from the two horns being attached to eight separate levers. With two doses of the standard and two of the test preparation the standard error of the estimate of potency was calculated to be less than 5 per cent in 18 out of 23 assays but in fact the true potency was known to be outside the calculated limits in ten instances. Holton³⁸ used rat uterus in the more usual type of test giving doses each of standard and unknown and obtained standard errors of about 3 per cent but little is known about the differential actions of vasopressin and oxytocin in such a test (see also Burn¹).

Antidiuretic Activity. Tests for estimating antidiuretic potency are described by Burn¹ and in the British Pharmacopoeia. Both use the rat and are based on the original work of Burn³⁹. The test depends on the comparison of the times elapsing until a maximum rate of excretion of urine is reached after the administration of water by stomach tube and

extract of the posterior lobe by injection. Groups of four rats were used in a cross over technique the rats which had received the standard on one day receiving the unknown on the next day and *vice versa*. Potency was calculated however with reference to a standard curve which was based on sixty four rats its position but not its slope being corrected in each test from the results obtained with the standard. The curve looked as though a linear log dose response relationship held but no work based on this assumption is known. It is not possible to estimate the error of the test. Burn states that eight comparisons yielded results within 23 per cent of the known value the average error being 12 per cent. Until the assay is put on a more satisfactory basis which allows of the computation of error from the internal evidence of the test or until many comparative assays are made by the present method this statement is the best that can be offered although much exploratory work has been undertaken. Thus Ham and Landis⁴⁰ investigated various factors and concluded that under certain conditions the estimation of total chloride excretion is preferable to measurements of urine volume but did not use modern statistical methods in designing their procedure or evaluating their results.

Pressor Activity The cat method for estimating pressor activity remains the only test which can be put forward at the present time. The extract of the posterior lobe is injected into a vein of a decapitated cat but successive doses cannot be given frequently usually not more often than once every thirty minutes sometimes not more often than once an hour. The rise in blood pressure which follows a constant dose decreases with successive injections at any rate if the injections are made more frequently than about once an hour. This is an unsatisfactory test for only a few readings can be made with each cat and no statement is possible about limits of error. The dog can be injected more frequently about once every fifteen minutes and Stewart⁴¹ reports that it is always possible to distinguish differences of 20 per cent. The rat behaves similarly and Landgrebe *et al*⁴² may be consulted for the methods employed which appear to discriminate between 10 per cent dosage differences. It would seem that rat methods are worth further investigation.

ADRENALS

Adrenal Cortical Hormones There are no international standards for the hormones of the adrenal cortex. They fall into two main groups those which resemble deoxycortone and those which resemble corticosterone. Of the first group deoxycortone appears to be the most potent substance for maintaining the life of adrenalectomised animals while of the second group 17 hydroxycorticosterone appears to be the most potent in affecting the metabolism of organic materials. Clinical interest centres at present on deoxycortone which is best

assayed by its effect on the survival time or weight gain of adrenalectomised rats or the maintenance of normal conditions in adrenalectomised dogs. No attempt will be made here to cover the field in its entirety since the subject is undergoing rapid development. A detailed review by Thayer⁴³ has been published.

Grollman⁴⁴ found that the survival of adrenalectomised animals is greatly influenced by the intake of sodium chloride and potassium ions. He also found the gain in weight under standard dietary conditions to be a better criterion than survival time in rats but Thayer could not obtain accurate results by that method. Thayer and his colleagues have not in fact found any test utilising survival, weight gain, sodium metabolism or renal function as measured by blood urea nitrogen which promises great accuracy. Thus the characteristic actions of deoxycortone have not yet been made the basis of an assay method which can be assigned any but very approximate and wide limits of error. Tests utilising a characteristic activity of corticosterone on the other hand attain considerable precision. Unfortunately this activity, the causing of an increase in glycogen or total fermentable sugar in the liver of adrenalectomised rats, is not exerted by deoxycortone at all.

In statistical comparison of the glycogen against total fermentable sugar test, Eggleston, Johnston and Dobriner⁴⁵ find that the latter is much more accurate. They injected adrenalectomised rats hourly for 7 hours and killed at 74 hours from the start of the test and then determined either the liver glycogen or total fermentable sugar. The log dose response line for the latter was much the steeper: each animal contributed about ten times as much information about relative potency as compared with those of the glycogen series. In a test with twenty mice on the standard and 20 on the unknown, the minimal limits of error ($P = 0.95$) to be expected in the two methods are

Glycogen	51 to 195 per cent
Total fermentable sugar	83 to 121 per cent

The error for the glycogen test would appear to be in line with that found by Dorfman, Ross and Shipley⁴⁶ who used a similar technique with adrenalectomised mice and referred the results of series of tests with only one dosage level to a standard curve. They found that when ten mice were used the error ($P = 0.95$) ranged from 50 to 250 per cent. The predicted error was 41 to 250 per cent. Their predicted error ($P = 0.95$) with forty mice per group (although all on one dose of the unknown) would thus appear to be about 60 to 160 per cent. However a similar method employing the estimation of liver glycogen gives lower errors in the hands of Venning, Hazman and Bell⁴⁷. These investigators modified the Reinecke and Kendall⁴⁸ test and gave injections of the hormones in 5 per cent glucose obtaining parallel log dose response lines for various cortical steroids and minimal expected limits of error ($P = 0.95$) of about 80 to 125 per cent for the hypothetical forty mouse test used here as a standard of comparison.

Adrenaline There is no international standard preparation of adrenaline. The substance may be extracted from adrenal glands or synthesised and characterised as a pure compound. For the purposes of standardisation a biological test is necessary, only to ensure that a sample has the full activity of pure adrenaline when the methods described by Burn¹ may be used. The blood pressure of the atropinised cat or dog gives very satisfactory estimates with standard errors of about 2 per cent⁴⁸ while the frog heart method⁵⁰ has about the same precision. In this latter test winter male frogs were found to be preferable and assays of the $x \rightarrow y \rightarrow y \rightarrow x$ type were used. It is a simple method and appears to be very useful when frogs of the right type are available although no data were given as to the relative errors of other than winter male frogs. It would seem to be unnecessary to attempt modifications of these tests which would give internal estimates of error so long as the laboratory performing them is regularly doing the same type of work and can keep a check on consistency. The limits of error ($P = 0.95$) can apparently be kept within about 95 to 105 per cent if care be exercised and the identity of the substance concerned is not usually in question.

THYROID

There is no international standard for thyroxine or thyroid preparations. The latter are usually assayed chemically on the basis that all the acid insoluble iodine is combined as thyroxine. However the recent use of artificially iodinated proteins has led to a revival of interest in biological tests because not all the acid insoluble iodine in such proteins is contained in thyroxine or other substances of similar activity.

Several methods for assaying thyroid preparations are given by Burn¹ none of them is very accurate and most are unwieldy. More recently two promising new tests (described below) have been devised for which details of limits of error can be given. The first one is an adaptation of the tadpole test first proposed by Lenhart⁵¹ the second is a mammalian test so far investigated with albino mice.

(i) *Xenopus Tadpole Method* This method was developed by Deanesly and Parkes⁵² who describe methods for obtaining and rearing the tadpoles at any season of the year. They decided to use eruption of the front legs as the response to thyroid activity. Groups of five tadpoles 18 to 25 millimetres in length are placed in 200 millilitres of distilled water to which the substance to be assayed is added as a fine suspension. The distilled water is changed to tap water after three days and the percentage of tadpoles showing eruption of one or both front legs is recorded on the seventh day. The slopes for thyroxine and various iodinated proteins are substantially the same but are affected by temperature steeper slopes being obtained at 24° than at room

temperature. At 24° the mean slope of nine tests was 0.95. With this slope a test using four groups of twenty tadpoles, two groups on the standard and two on the unknown, will have minimal limits of error ($P = 0.95$) of 78 to 128 per cent.

The biological activity of eight preparations of iodinated plasma arden or casein as measured by the tadpole test agreed very closely with the capacity of these preparations to stimulate milk yield in cows (Deanesly and Parkes⁵³). However diiodotyrosine is active in mammals so that some slight doubt is cast on the specificity of the test. This was one reason for the development of a new mammalian test.

(ii) *Enclosed Vessel Technique with Mice*. This method is described by Smith, Emmens and Parkes⁵⁴. Young male albino mice are injected with the substances under test on days 1, 3 and 5, and the test is performed two or three days after the last injection, that is on the seventh or eighth day. Each mouse is placed in a separate 2 lb glass jar with an air tight lid; the response is the survival time in minutes. This is decreased by increasing the temperature and by injections of thyroxine or an iodinated protein. The test is performed at 23°, which gives survival times of convenient length and about a minimal variance. The preliminary results with the test using male mice, which are less variable than females, indicate that with ten mice per group the minimal limits of error ($P = 0.95$) are about 73 to 137 per cent.

The peculiar feature of this mammalian test is that the early death of treated mice is not due to a rise in metabolic rate, even with more prolonged periods of treatment (up to three weeks), but to their increased sensitivity to anoxia or carbon dioxide poisoning. It has yet to be shown how highly the factors responsible for early death in this assay correlate with metabolic effects, and whether such a test is in fact to be preferred to the tadpole test.

PARATHYROIDS

There is no international standard for extracts of the parathyroid glands. Several test methods are described by Burn¹, but it is not at present possible to compare their relative accuracies. The two most promising methods would appear to be that utilising the effect of parathyroid extracts in diminishing the narcotic action of magnesium sulphate to mice, and that utilising the estimation of urinary calcium in the rat⁵⁵.

The potency of Parathyroid Injection U.S.P. XIV is assayed by its effect on the serum calcium of normal dogs and is expressed in terms of U.S.P. Parathyroid Units. Each unit represents one one hundredth of the amount of injection required to raise the serum calcium by 1 milligram per 100 millilitres within 16 to 18 hours of administration.

PANCREAS

Insulin (Ordinary Insulin) The international unit of insulin is the activity of 0.0455 milligram of the international standard (pure crystalline insulin 1935) preparation. The new unit was defined in relation to the 1925 crude standard of which the unit was 0.125 milligram.

The two standard methods for the assay of insulin—the mouse convulsion method and the rabbit blood sugar method—are outlined below. Details for the laboratory procedure and general references are given by Burn¹ and for a modified mouse convulsion method by Young and Lewis⁵⁶.

(1) *Mouse Convulsion Method* This uses a quantal response, namely the proportion of mice exhibiting convulsions after injection with insulin under standard conditions. The mice are placed in a thermostatically controlled cabinet at between 29° and 37°, usually at 32° in modern work. Details of a standard procedure are given in the British Pharmacopoeia. The error of this method was investigated by Irwin⁵⁷ who found a mean slope of 3.0 in the series of assays with which he dealt and reports fully on expected errors and the actual fiducial limits of error found in the tests. A slope of 3.0 is low; according to present standards, the mean of a series of slopes found in the laboratories of one insulin producer in the course of recent routine work was 5.3. With Irwin's data, the minimal limits of error ($P = 0.95$) to be expected with a test using forty mice for each sample are 62 to 162 per cent, with a slope of 5.3 these limits are 76 to 132 per cent. The standardisation of batches of insulin is normally carried out with a total of several hundred mice for each batch; neither the manufacturers nor the licensing authorities would be content with fiducial limits of error ($P = 0.95$) wider than about 85 to 115 per cent, and they are usually expected to be within 90 to 110 per cent of the mean estimate of potency.

(2) *Rabbit Blood Sugar Method* This method depends on the reduction in the blood sugar level of rabbits which follows the injection of insulin. As at present used, it involves the measurement of the initial blood sugar level and the hourly blood sugar level, or the blood sugar level of pooled hourly samples for five hours subsequently. Full details of a standard procedure are given in the British Pharmacopoeia and of the various laboratory reagents and techniques by Burn¹. The British Pharmacopoeia procedure uses the cross over design elaborated by Marks and his co-workers (see Marks and Pak⁵⁸). In this test, the same rabbits are used with the standard and unknown on different days, so that differences in the mean sensitivity of individual rabbits are segregated in the analysis of the test and do not contribute to the estimate of error. The form of test recommended is the twin cross over test, full statistical examination of which is due to Smith, Marks, Fieller and

Broom⁶⁵ Four groups of rabbits are used and high and low doses of the standard and unknown are each administered to one of these groups on the first day of the test. On the second day of the test the group which received the high dose of the standard receives the low dose of the unknown while the group which received the low dose of the standard receives the high dose of the unknown. The other two groups are crossed over in a similar fashion.

The error of this type of test was investigated by Pieller, Irwin, Marks and Shrimpton⁶⁶ and in various papers by Bliss and Marks.⁸ These authors agree remarkably well in that although Bliss and Marks found higher values for the slope their error mean squares are also higher and estimates of limits of error from the two series are almost identical. If the standard test with twenty animals for each sample is modified in view of the cross over procedure to ten animals for each sample five animals a group in a twin cross over test and two observations per animal the expected minimal limits of error ($P = 0.95$) are 82 to 122 per cent.

Delayed Action Insulins Globin zinc insulin and protamine zinc insulin have a more prolonged action than ordinary insulin the degree of prolongation being greater with protamine zinc insulin. There are at present no standard preparations of these modified insulins which are therefore compared with ordinary insulin using the rabbit test. The test of the British Pharmacopœia requires that when the average blood sugar of the rabbits receiving the standard preparation has just returned to the initial level as judged graphically, that of the rabbits receiving protamine zinc insulin should not be more than 80 per cent of the corresponding initial value. This is not an exact method of comparison and the setting up of suitable standards for the delayed action insulins is in progress which will make possible the performance of tests giving precise estimates of potency and limits of error without reference to the present standard preparation. It should be noted that the amount of ordinary insulin used in preparing the delayed action forms is carefully controlled so that the present tests are tests of the delaying factor not of the unitage in terms of the international standard preparation for ordinary insulin.

GONADS

Oestrogens There are two international standard preparations for the assay of oestrogenic activity oestrone and oestradiol monobenzoate. The international unit is in each case the activity of 0.0001 milligram of the standard preparation. The oestrone standard is intended for comparison with non esterified oestrogens and the oestradiol monobenzoate standard for comparison with benzoylated preparations. It has been shown however that with the usual test methods it is

valid only to compare oestrone with oestrone and oestradiol monobenzoate with oestradiol monobenzoate since the influence of the route of administration the solvent the test-object and the particular test method used is so great that even pure crystalline steroids cannot usefully be compared with one another.⁴¹ This has led to no great difficulties in practice because the oestrogens are usually prepared as pure substances and can be characterised physically. The potency of such a preparation may therefore be checked if necessary by reference to the standards or to another pure sample of the compound being examined if it is not oestrone or oestradiol benzoate.

The most frequently used assay in standardisation is the Allen Dowsy test for vaginal cornification in the mouse or rat.⁴² This test exists in various modifications the most useful of which would seem to be the injection of the substances under test in one or two daily doses in oily solution preferably in mice and the taking of vaginal smears twice a day on the third and fourth days of the test. A smear with no leucocytes and with cornified epithelial cells is scored as positive all others as negative the response is therefore quantal. There is wide general agreement that the value of the slope of the log dose response line is (with few exceptions) between 5 and 6. If a slope of 5.5 is found the minimal limits of error ($P = 0.95$) to be expected in a test with forty mice per substance are 77 to 130 per cent.

Various other tests exist many founded on the increase in uterine weight of immature or spayed animals after injection of oestrogens. Of these the Astwood six hour test⁴³ is of interest because of its speed. Immature female rats twenty-one to twenty-three days old are injected with the oestrogen and the uterus weighed six hours afterwards. This method seems quite accurate but unfortunately the data are not presented in such a form as to allow of the estimation of the errors of the test.

Progesterone. The international unit of progestational activity is that contained in 1.0 milligram of international standard progesterone.

There is no method of assaying progesterone biologically from which sufficient data are available for estimating errors. The McPhail test⁴⁴ gives arbitrary grades to the degree of progestational proliferation of the uterus of the oestrogen primed rabbit. This test could probably be made the basis of a successful quantal or even graded response assay but since it involves histological examination of the uterus it would always be rather laborious. It is described by Burn.⁴ Another promising test is that of Astwood⁴⁵ which utilises the deciduoma reaction in the rat and could presumably also be adapted for precise work. There is no general demand for such tests however because crystalline progesterone is now produced commercially and the excretion product in human urine pregnanediol is estimated chemically.

Androgens The international unit of androgenic activity is that exhibited by 0.1 milligram of international standard androsterone. The same situation as discussed above with reference to the oestrogens is found when dealing with androgens. There is no commercially prepared androsterone for clinical use; the compound used almost exclusively in medicine is testosterone propionate for which no biological standard exists or is needed and which cannot be directly compared with androsterone. The practical need for standardisation of androgens is therefore very limited.

The introduction of growth in the atrophic comb of the capon was the only test recognised by the 1935 Conference on the Standardisation of Sex Hormones⁶⁶ as being sufficiently specific and precise. As usually performed, the test requires the injection of the androgen for three to five days. The response is the increase in comb size measured in various ways by different investigators. A detailed investigation of the test by Emmens showed that when the response is the added increase in length plus height of the comb it is linearly related to dose (not log dose) and that the variance increases with the age of the birds. Basing an estimate on the average standard deviation in the three day tests, an assay with twenty birds per substance would have minimal expected limits of error ($P = 0.95$) of about 62 to 138 per cent. These limits should be reduced by using a cross over technique and further reduced if a common zero slope ratio method of assay could be employed.

The capon comb test, although undoubtedly the most appropriate of all tests available for standardisation, requires facilities often not available to the bio assayist. Various tests employing the immature or castrated rat or mouse are current; the responses most frequently used are the weight increases of seminal vesicles or prostate glands under androgenic stimulation. Relatively rapid tests utilising seminal vesicle weight seem to be as accurate as the more prolonged tests first evolved and two recent examples are of interest. Greene and Burrill⁶⁷ use immature male rats twenty to twenty-two days old and inject once with androgen. Forty-eight hours later the seminal vesicles are dissected and weighed fresh. The log dose response relationship is linear from 5 to 50 micrograms of testosterone propionate and calculations from Greene and Burrill's data indicate that the minimal expected limits of error ($P = 0.95$) are 64 to 157 per cent in a forty rat test of the usual design. Mathieson and Hays⁶⁸ used castrated young male rats in a similar test. Their data indicate that the limits of error in a forty rat test would be 74 to 136 per cent as long as differences between groups of rats castrated at the same time are, if necessary, segregated in the analysis of variance. In two practical examples using only thirty-two rats per assay the limits of error ($P = 0.95$) were found to be 70 to 140 per cent approximately.

RELATIVE ACCURACY OF TEST METHODS

The approximately minimal numbers of animals required to give an accuracy of 80 to 125 per cent at the 95 per cent level of significance in different tests in which groups of animals are used are shown in Table XII

TABLE XII

Approximate total numbers of animals (not litter mates) needed to achieve minimal limits of error ($P=0.95$) of 80 to 125 per cent in various assays

Substance	Animal	Method	No required
Thyrotrophic hormone	Guinea pig	Thyroid weight ^{1,2}	50
Lactogenic hormone	Pigeon	Crop gland weight ³	65
Growth hormone	(a) Plateaued rat (b) Hypophysectomised rat	Body weight	110
Adrenocorticotrophic hormone	(a) Chick (b) Hypophysectomised rat	Body weight ⁴	220
		Adrenal weight	35
		Adrenal weight ⁵	240
Chorionic gonadotrophin	(a) Rat (b) Mouse	Ovarian weight ^{6,7}	90
Serum gonadotrophin	Rat	Vaginal smear ⁸	45-130
Adrenocortical extracts	(a) Rat (b) Rat (c) Rat	Ovarian weight	110
		Liver glycogen	350
		Total fermentable sugar	30
		Liver glycogen	40
Thyroxine and iodoproteins	(a) Tadpole (b) Mouse	Eruption of front legs	95
Insulin	(a) Mouse (b) Rabbit	Survival time	80
		Convulsions	120
Estrogens	Mouse or rat	Fall in blood sugar ^{9,10}	16*
Androgens	(a) Capon (b) Rat	Vaginal smear ^{4,11}	110
		Comb growth ⁹	130
		Seminal vesicle weight ¹²	75-130

Cross over test

These numbers are directly proportional to the inherent precision of various methods of assay as estimated from available data. It should be noted that the sixteen rabbits required on the average in insulin assays are each used twice in the cross over technique and that the precision of this method is greatly enhanced by the elimination of differences between the mean sensitivity of individual rabbits in the assay. The accuracy of the assays of gonadotrophins (and doubtless other substances) is also much improved if litter mates can be used and the corresponding litter differences eliminated from the estimate of error. Thus the most accurate of this group of tests when litter mates are used would seem to be the assay of serum gonadotrophin by the method employing rat ovarian weight. It is rarely advisable to use less

than twenty animals in an isolated assay, however, because the fiducial limits of error widen rapidly with small numbers of observations

REFERENCES

- 1 BURN J H *Biological Standardisation* 1950 Oxford University Press London
- 2 EMMENS C W *Spec Rep Ser med Res Coun Lond* No 234 1939 H M S O London
- 3 GADDUM J H *Biochem J* 1931 25 1113
- 4 BLISS C I *Industr Engng Chem* 1941 13 84
- 5 BLISS C I and MARKS H P *Quart J Pharm* 1939 12 82 182
- 6 GADDUM J H *Spec Rep Ser med Res Coun Lond* No 183 1933 H M S O London
- 7 BLISS C I *Ann appl Biol* 1935 22 134
- 8 FISHER R A and YATES F *Statistical Tables* 3rd Edition 1948 Oliver and Boyd Edinburgh
- 9 EMMENS C W *Principles of Biological Assay* 1948 Chapman and Hall London
- 10 IRWIN J O *J Hyg Camb* 1943 43 121
- 11 *Quart Bull Hlth Org L o N* 1938 7 887
- 12 ROWLANDS I W and PARKES A S *Biochem J* 1934 28 1829
- 13 BERGMAN A S and TURNER C W *Endocrinology* 1939 24 656
- 14 RIDDLE O BATES R W and DYKSTROM R W *Amer J Physiol* 1933 105 191
- 15 EMMENS C W *J Endocrinol* 1940 2 194
- 16 REECE R P and TURNER C W *Bull Mo agric Exp Sta* 1937 266 1
- 17 MEITES J BERGMAN A J and TURNER C W *Endocrinology* 1941 28 707
- 18 HALL S R *Endocrinology* 1944 34 1
- 19 BLISS C I and ROSE C L *Amer J Hyg* 1940 31 79
- 20 EMMENS C W *Bull Hlth Org L o N* 1938 8 901
- 21 MARX W SIMPSON M E and EVANS H M *Endocrinology* 1942 30 1
- 22 GRIFFITHS M and YOUNG F G *J Endocrinol* 1942 3 96
- 23 EVANS H M SIMPSON M E MARX W and KIBRICK E *Endocrinology* 1943 32 13
- 24 MOON H D *Proc Soc exp Biol N Y* 1937 35 649
- 25 MOON H D *Proc Soc exp Biol N Y* 1940 43 42
- 26 BATES R W RIDDLE O and MILLER R A *Endocrinology* 1940 27 781
- 27 FINNEY D J *Quart J Pharm* 1945 18 77
- 28 SIMPSON M E EVANS H M and LI C H *Endocrinology* 1943 33 261
- 29 SAYERS G SAYERS M A TEAN YING L and LONG C N H *Endocrinology* 1946 38 1
- 30 REISS M and HALKERSTON I D K *J Pharm Pharmacol* 1950 2 236
- 31 EMMENS C W *Bull Hlth Org L o N* 1939 8 862
- 32 DEANESLY R *Quart J Pharm* 1935 8 651
- 33 HEARD R D H and WINTON S S *J Physiol* 1939 96 248
- 34 EMMENS C W *Bull Hlth Org L o N* 1939 8 887
- 35 GADDUM J H *Quart J Pharm* 1938 11 697
- 36 HAMBURGER C *Acta Pharmacol Toxicol* 1945 1 112
- 37 BACHINSKY W M ALLMARK M G and MORRELL C A *Canad J Res E* 1945 23 126
- 38 HOLTON P *Brit J Pharmacol* 1948 3 328
- 39 BURN J H *Quart J Pharm* 1931 4 517
- 40 HAM G C and LANDIS E M *J cl Invest* 1942 21 455
- 41 STEWART G A *J Pharm Pharmacol* 1949 1 436
- 42 LANDGREBE F W MACAULAY M H I and WARING H *Proc roy Soc Edinb Sec B* 1946 62 202
- 43 THAYER S A *Vitamins and Hormones* 1946 Edited by R S Harris and K V Thimann Academic Press New York Volume 4 page 311
- 44 GROLLMAN A *Endocrinology* 1941 29 855 862
- 45 EGGLESTON N M JOHNSTON B J and DOBRINER K *Endocrinology* 1946 38 197
- 46 DORFMAN R I ROSS E and SHIPLEY R A *Endocrinology* 1946 38 178

- 47 VENNING H KAZMIN V E and BELL J C *Endocrinology* 1946 38 79
- 48 REINECKE R M and KENDALL E C *Endocrinology* 1942 31 573
- 49 THOMPSON H E *J Amer Pharm Ass* 1945 34 2b5
- 50 WEST G H *J Physiol* 1943 102 367
- 51 LENHART C H *J exp Med* 1915 22 739
- 52 DEANESLY R and PARKES A S *J Endocrinol* 1945 4 324
- 53 DEANESLY R and PARKES A S *J Endocrinol* 1945 4 356
- 54 SMITH A U EMVENS C W and PARKES A S *J Endocrinol* 1945 5 186
- 55 DYER F J *Quart J Pharm* 1933 6 426
- 56 YOUNG H M and LEWIS A H *Science* 1947 100 368
- 57 IRWIN J O *Quart J Pharm* 1943 16 352
- 58 MARKS H P and PAK C *Quart Bull Hlth Org L.N. Special No November*
1936 631
- 59 SMITH K W MARKS H P FIELLER H C and BROOM W A *Quart J Pharm*
1944 17 108
- 60 FIELLER H C IRWIN J O MARKS H P and SHUMPTON H A G *Quart J*
Pharm 1939 12 724
- 61 PEDERSEN BJERGAARD K *Comparative Studies Concerning the Strengths of*
Oestrogenic Substances 1939 Oxford University Press London
- 62 ALLEN E and DOISY E A *Physiol Rev* 1927 7 600
- 63 ASTWOOD E B *Endocrinology* 1938 23 25
- 64 MCPHAIL M K *J Physiol* 1934 83 145
- 65 ASTWOOD E B *J Endocrinol* 1939 1 49
- 66 *Quart Bull Hlth Org L.N.* 1935 4 Extract 10
- 67 GREENE R R and BURRILL H W *Endocrinology* 1941 29 402
- 68 MATHIESON D and HAYS H W *Endocrinology* 1945 37 275
- 69 *British Pharmacopoeia* 1948

CHAPTER VI

ACTION AND USES

DURING recent years the action and uses of hormones have been studied extensively using the standardised extracts and pure natural and synthetic compounds now available to the clinician. Since hormone preparations do not necessarily produce the same effects in patients as they do in experimental animals their clinical use is frequently empirical. The treatment of glandular underactivity consists in the administration of the correct amount of a standardised extract or synthetic compound to make good the deficiency. Overactivity of a gland is corrected by the surgical removal of a part of the gland or by administering preparations which have an antagonising action for example thiouracil in hyperthyroidism. Treatment with hormones must be regulated for individual patients since the correct dosage necessarily depends on the degree of hormone production of the deficient gland and on the degree of activity of other interrelated glands. Hormones from the anterior lobe of the pituitary stimulate many other glands and large doses of oestrogens and other hormones can depress the activity of the pituitary. The complicated interaction of the different glands and the variable response to therapy impose difficulties and limitations in the way of satisfactory treatment with hormones.

Before commencing hormone therapy, an accurate diagnosis is essential and local causes of disease must be excluded. Treatment with hormones can be curative only if the diseased gland is capable of functioning on discontinuation of therapy. Most hormone preparations however act by substitution only; they do not have a permanent effect and the fact that they can inhibit the hormone production of other glands must be taken into consideration. After the prolonged administration of some hormone preparations their further use is followed by a marked reduction or even absence of the physiological response. The doses of hormone preparations should therefore be sufficient to correct the deficiency only since excessive doses may make any subsequent treatment relatively ineffective. Collip and Anderson¹ showed that laboratory animals became non responsive to the thyrotrophic hormone after prolonged treatment and if the blood of these non responsive animals was injected into untreated animals these also did not respond to injections of the thyrotrophic hormone owing to the presence of anti hormones. Clinically it is known that the continued use of some hormone preparations particularly those of a protein nature such as the gonadotrophins can result in a state refractory to further injections. Leatham and Rakoff² showed that the antihormones which develop following the administration of pregnant mares' serum gonadotrophin are hormone specific and with the appearance of the hormone inhibition treatment should be suspended or a gonadotrophin from another

source should be administered Zondek³ considers that antihormones are unlikely to occur with the steroid hormones or insulin

THYROID

The thyroid gland intensifies the action of the intracellular metabolic enzymes thus increasing tissue metabolism. It has a specific affinity for iodine and this element in addition to the thyroid hormone thyroxine must be considered in the treatment of thyroid disorders. A deficiency of iodine in the diet produces an enlargement of the thyroid gland. This condition is sometimes known as endemic goitre because of its frequent occurrence in districts in which there is a deficiency of iodine in the soil and water. Simple goitre can be prevented by giving small doses of iodine in the form of potassium or sodium iodide which is usually added to common salt in the proportion of 1 part in 5000 to 20 000 of salt. Treatment with iodine itself is not so satisfactory but some workers have obtained beneficial results with small doses of thyroid.

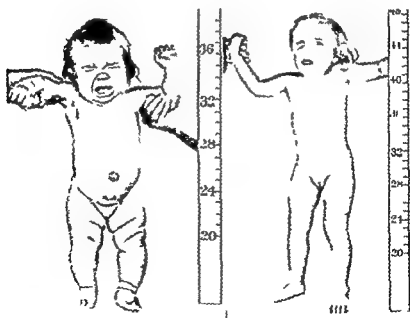


FIG. 25

CRETINISM

Due to congenital deficiency of thyroxine. The patient is shown at 20 months (a) just before the beginning of treatment by the continuous administration of thyroxine and at 3 years (b).

Hypothyroidism A deficient secretion of the thyroid hormone, or hypothyroidism may occur at any age and may be due to injury of the gland either by accident or disease. Occasionally it may occur after the removal of too much of the gland in operations for hyperthyroidism. In children the thyroid gland may be deficient from birth when the disease is known as cretinism; if however, thyroid deficiency occurs in young children it is called infantile myxœdema.

The symptoms of cretinism which may not be observed immediately are a slow gain in weight, retarded mental development and a disturbance in bone formation which can be demonstrated radiologically.



FIG. 26

TYPICAL MYXŒDEMA

Due to underactivity of the thyroid. Hair brittle and coarse, skin puffy and dry, general lethargy due to slowing down of the metabolic processes.

Cretins are always dwarfed persons with a sallow complexion, puffy eyelids and a dry skin. Thyroid if given early promotes normal growth and physical development but mental development frequently remains retarded owing to the damage to the brain which has taken place during embryonic life. The dose of thyroid must be adjusted to the individual needs of the patient; approximately 30 milligrams ($\frac{1}{2}$ grain) daily may be sufficient for infants and 60 milligrams (1 grain) or more for children of four to twelve years of age. Normal growth can be restored in infantile myxœdema if treatment is given early.

Thyroid deficiency in adults or myxœdema was first described by

Gull in 1873 and is sometimes known as Gull's disease. The name myxœdema (mucous œdema) was given to it by Ord in 1878. Persons with hypothyroidism usually have a placid drowsy expression with slow speech and intellectual processes. The features are coarse and the skin thick. In contrast to persons with hyperthyroidism they have a small appetite and constipation is common. The treatment of myxœdema with thyroid produces excellent results and the effects of treatment appear within a week. As with all other hormone deficiency states the dose required varies considerably with the patient. Treatment is commenced with a dose of 60 milligrams (1 grain) daily and this is gradually increased until the symptoms disappear. A dose of 0.2 gramme (3 grains) daily is sufficient for most patients but larger doses may be required. It is important to increase the dose slowly because the thyroid hormone requires at least twenty-four hours to take effect and cumulative effects may occur. To guard against these thyroid therapy is sometimes omitted every fourth week. Treatment is best controlled by estimation of the basal metabolic rate or more easily by determination of blood cholesterol⁴.

Thyroxine is sometimes administered in the treatment of hypothyroidism as thyroxine sodium but this appears to have no advantages when given by mouth over thyroid preparations. Thyroxine sodium which is available in tablets containing 0.05 and 0.1 milligram equivalent respectively to about $\frac{1}{4}$ and 1 grain of thyroid is more active than D-thyroxine sodium and is therefore preferable to the racemic thyroxine sodium described in the British Pharmacopœia 1932. Thyroxine sodium is also administered by intravenous injection as an aqueous solution. As with thyroid treatment with thyroxine sodium should be controlled by determining the basal metabolic rate or blood cholesterol.

Hyperthyroidism The essence of treatment is to control the overactivity of the gland either by the surgical removal of a part of the gland or by drug therapy. Iodine is frequently given before operations for removal since it lowers the basal metabolic rate and temporarily reduces the size of the goitre. The mechanism of the action of iodine in hyperthyroidism is not clearly understood.

Interest in drugs which can inhibit thyroid activity was stimulated by the observation that the administration of thiourea derivatives to laboratory animals was followed by typical signs of thyroid deficiency⁵ and hundreds of different compounds have been prepared and tested in this respect⁶. These compounds act by blocking the uptake of free iodine in the thyroid cells before the iodination of tyrosine. The first of these compounds to be used clinically was thiourea but this has been replaced by the more active and less toxic derivatives thioracil, methylthioracil and propylthioracil.

The administration of antithyroid drugs leads to the following series

of events (a) the production of thyroxine by the thyroid gland is decreased (b) the control of the pituitary gland by thyroxine is diminished (c) increased amounts of the thyrotrophic hormone are excreted by the thyroid gland and (d) the thyroid gland becomes hyperplastic. As a result the thyroid gland remains enlarged although it is not producing an abnormal amount of thyroxine.



FIG. 27

TYPICAL THYROTOXICOSIS

(Exophthalmic Goitre, Graves Disease)
Due to overactivity of the thyroid. Eyes
bulging, general state of anxiety and
restlessness.

There is a close interrelationship between hormones and vitamins and an antagonism between vitamin A and tyrosine is known. Simkins⁷ used vitamin A in massive doses to treat two patients with hyperthyroidism and stated that he considered it a promising therapeutic agent for this condition.

Certain chemotherapeutic agents can control hyperthyroidism but they rarely cure the condition. Surgery has the advantage that a cure can be quickly obtained. A useful comparison of the results obtained with antithyroid drugs is given by Williams⁸. The most potent preparations were propylthiouracil and cyclopropylthiouracil and Williams considers that they are the most desirable ones to use.

Radioactive iodine has been used to apply radiations directly within the cells of the thyroid gland of patients with hyperthyroidism and there is no doubt that it is an effective way of producing a remission but an appraisal of its value as compared with other methods cannot be made until more patients have been treated over a long period

Other Uses of Thyroid Thyroid has been recommended for many conditions not directly associated with an underactive thyroid gland Obesity although seldom evidence of glandular disorders is not necessarily due to hypothyroidism and successful treatment with thyroid may be due to its diuretic action Thyroid has also been used in the treatment of a variety of gynaecological conditions such as amenorrhoea sterility abortion and menstrual disorders and there may be some justification for its use in these conditions because it is known that there is a close interrelationship between the gonads and the thyroid gland

Thyroid Preparations Murray* who first used thyroid in the treatment of myxoedema gave a glycerin extract by subcutaneous injection It was soon discovered however that thyroid preparations were effective by mouth and accurately standardised preparations of thyroid have been available for some time The potency of different preparations is usually estimated from the amount of iodine which they contain as thyroxine but biological assays are also employed by some manufacturers The doses of thyroid in the above sections refer to the powdered defatted standardised thyroid of the British Pharmacopoeia and not to the fresh gland (see page 183)

PARATHYROID

The surgical removal of the parathyroid glands produces tetany This is due to a reduction in the blood calcium the symptoms of which can be relieved by the intravenous or oral administration of calcium salts They can also be relieved by the administration of an extract of the parathyroid glands which produce a hormone capable of use in replacement therapy The parathyroid hormone plays an important part in regulating the calcium level in the blood bones and urine but the mechanism of its action is still debatable

Hypoparathyroidism Extreme hypofunction of the parathyroid glands produces hyperexcitability of the nervous system with painful tonic spasms of the muscles The hands wrists and ankles are usually affected first spasms occurring later in the face trunk and sometimes the laryngeal muscles Tetany may be latent although typical symptoms are not present muscular spasms can be induced in susceptible individuals by constricting the upper arm with a tourniquet when a typical spasm in the hand is produced This is known as Trousseau's

of events (a) the production of thyroxine by the thyroid gland is decreased (b) the control of the pituitary gland by thyroxine is diminished (c) increased amounts of the thyrotrophic hormone are excreted by the thyroid gland and (d) the thyroid gland becomes hyperplastic. As a result the thyroid gland remains enlarged although it is not producing an abnormal amount of thyroxine.



FIG. 27

TYPICAL THYROTOXICOSIS

(Exophthalmic Goitre, Graves Disease)
Due to overactivity of the thyroid. Eyes
bulging, general state of anxiety and
restlessness.

There is a close interrelationship between hormones and vitamins and an antagonism between vitamin A and tyrosine is known. Simkins⁷ used vitamin A in massive doses to treat two patients with hyperthyroidism and stated that he considered it a promising therapeutic agent for this condition.

Certain chemotherapeutic agents can control hyperthyroidism but they rarely cure the condition. Surgery has the advantage that a cure can be quickly obtained. A useful comparison of the results obtained with antithyroid drugs is given by Williams.⁸ The most potent preparations were propylthiouracil and cyclopropylthiouracil and Williams considers that they are the most desirable ones to use.

Calciferol in Tetany For the treatment of patients with chronic tetany calciferol (vitamin D) has been employed although its action on calcium and phosphorus metabolism is different from that of the parathyroid hormone. Shelling¹ states that therapeutic amounts of calciferol invariably cause a positive balance of calcium and phosphorus and produce healing in the metaphyses of the bones if rickets be present whereas parathyroid hormone causes a negative calcium balance and a retardation in ossification. Schultz and Christensen¹³ used large doses of calciferol to treat eleven patients suffering from parathyroid tetany and found that it had an effect on calcium metabolism similar to that of dihydrotachysterol. The preparation used was an oily solution containing 300 000 units of calciferol equal to 7.5 milligrams in 1 millilitre. Subsequent patients were given 5 milligrams of calciferol daily supplemented by intravenous injections of calcium lactate during the first few days. There was a prompt rise in the serum calcium concentration but from one to five months elapsed before the maximal effect of calciferol was obtained. When adjusted the patients were just as well under treatment with calciferol as with dihydrotachysterol and showed no signs of tetany or other morbid conditions. The final daily dose of calciferol was from 1.5 to 4.5 milligrams and the concentration of serum calcium in all patients after adjustment 9 to 11.5 milligrams in 100 millilitres. Although the symptoms of tetany may be alleviated with either calciferol or parathyroid hormone it is only the former which increases the amount of calcium in the body.

Hyperparathyroidism An excessive secretion of the parathyroid hormone results in an increased amount of calcium in the blood serum and a decrease of phosphorus. Calcium is withdrawn from the bones which become cystic and deformed. Von Recklinghausen¹⁴ first described the bone disorder which is also known as generalised osteitis fibrosa cystica. It is an advanced stage of hyperparathyroidism characterised by gradual rarefaction and softening of the bones with development of cystic areas and deposition of calcium in soft tissues. Hyperparathyroidism can be produced experimentally by continued overdosage of the parathyroid hormone but the cause of the overactivity is not known. A deficiency of calciferol is thought to stimulate hyperplasia of the parathyroids with resulting adenomata which may or may not produce the hormone. If they do symptoms of hyperparathyroidism result. Treatment which consists of the surgical removal of the tumour usually gives relief. Keating and Cook¹⁵ in an analysis of twenty-four cases showed that in only seven was there classic evidence of disease of the bones but calcification of the kidneys or renal calculi occurred in twenty-two of the patients. They emphasise that in hyperparathyroidism involvement of the urinary tract is a more common and important symptom than bone involvement and they suggest that every patient who has renal calculi should be suspected of having

sign and is used as a diagnostic test. Other diagnostic tests are based on Chvostek's sign in which a slight momentary twitch is obtained by tapping the cheek over the facial nerve in front of the ear with a percussion hammer and Erb's sign in which there is increased irritability brought about by electrical stimuli. Hypoparathyroidism is always accompanied by numbness of the extremities.

One of the most reliable diagnostic tests is the decrease in the concentration of calcium ions in the blood serum. The normal serum calcium level is 10 milligrams per 100 millilitres; in tetany it may be 4 to 6 milligrams per 100 millilitres, but the severity of symptoms does not always vary in proportion to the level of serum calcium. Although the phosphorus content of the blood may remain unchanged, the ratio of phosphorus to calcium is altered so that the amount of phosphorus is relatively increased.

The administration of the parathyroid hormone alone is not a satisfactory treatment for most patients with parathyroid deficiency, and it is usually used as an adjuvant to other forms of therapy. The parathyroid hormone is ineffective when given by mouth, and its effects by injection are cumulative. The subcutaneous injection of 1 to 3 millilitres (100 to 300 units) of a preparation such as Parathyroid Injection (see page 176) usually results in raising the serum calcium to normal for a period of eight to eighteen hours immediately following the injection.¹⁰ For the treatment of tetany, calcium salts and dihydrotachysterol and calciferol with or without the parathyroid hormone are also employed. The intravenous injection of a 10 per cent solution of calcium gluconate causes an immediate rise in the calcium concentration in the serum, but this rise is not maintained. There is a slower but more prolonged effect with the parathyroid hormone, but there is also a gradual loss of effect following continued treatment, probably due to the formation of antihormones in the blood.

Dihydrotachysterol in Tetany. Dihydrotachysterol (AT 10) was first observed by Holtz in 1934 to exert an antitetanic action. It is used in the form of a 0.5 per cent solution in oil, the dose being from 1 to 2 millilitres (15 to 30 minims) by mouth at the commencement of treatment with smaller doses for maintenance. MacBryde¹¹ reported that a normal blood calcium level was maintained in seven patients over a period of three months to a year with doses of from 0.3 to 1 millilitre (5 to 15 minims) of dihydrotachysterol solution daily, supplemented by 4 to 10 grammes (60 to 150 grains) daily of calcium lactate or calcium gluconate. During treatment frequent estimations of serum calcium are necessary to avoid hypercalcaemia, and regulation of the diet to contain a high intake of calcium and a low intake of phosphorus is considered important by some authorities. Foods which should be avoided to maintain a low phosphorus intake include milk, meat, cheese, potatoes and cocoa.

readjustment of the equilibrium and there is probably an increased supply of adrenaline which has an antagonising action on insulin

Diabetes Mellitus : Hypo insulinism or diabetes mellitus the classical symptoms of which are polyuria and loss of weight is a comparatively common illness and may develop at any age. Many authorities stress obesity as a predisposing factor. Patients with sugar in their urine are not necessarily diabetic. The condition can be diagnosed and differentiated from renal glycosuria and other non diabetic conditions by means of the glucose tolerance test and an estimation of the amount of sugar in the blood (see page 22). Marble¹⁸ points out that patients with benign conditions may be incorrectly diagnosed as having diabetes mellitus. He finds pentosuria and fructosuria to be comparatively rare harmless conditions having no relation to diabetes mellitus and describes procedures for a differential diagnosis.

Insulin Preparations : Attempts to produce insulin compounds which are not destroyed by the digestive enzymes in the stomach have proved unsuccessful and although claims have been made for substances which are active by mouth insulin preparations for parenteral use remain the only therapeutically effective substances for use in the treatment of diabetes mellitus. Three types of preparations are available. Injection of insulin (*Injectio Insulini B P*) is a sterile solution of crystalline insulin hydrochloride containing 20, 40 or 80 units per millilitre. To avoid confusion it is sometimes called ordinary regular soluble or unmodified insulin. Injection of protamine zinc insulin (*Injectio Insulini Protaminati cum Zinco B P*) introduced in 1936 is a sterile suspension of a compound of insulin and a suitable protamine with the addition of a trace of zinc chloride. It contains 40 or 80 units per millilitre. The zinc chloride increases the stability of the preparation and delays the action of the insulin probably by interfering with an enzymatic process concerned in the separation of insulin from the insulin protein precipitate which is formed in the tissue fluids. Globin zinc insulin introduced in 1940 is a clear solution of a compound of insulin and globin, a protein obtained from hæmoglobin with the addition of a trace of zinc chloride. Mixtures of injection of insulin and injection of protamine zinc insulin are also employed. Patients react to the different insulin preparations in a uniform manner but they vary in the amount required to produce similar responses owing to differing sensitivities to insulin.

Action of Different Insulin Preparations The action of ordinary insulin is rapid in onset and a marked lowering of the blood sugar level occurs soon after injection giving immediate relief from the symptoms of hyperglycæmia. Insulin is quickly absorbed from the site of injection through the lymphatics into the blood stream. The

hyperparathyroidism Rogers¹⁶ described two patients with hyperparathyroidism not recognised during life and without a history suggesting osseous or renal disease. The first patient had an adenoma of the parathyroid gland and the second hyperplasia of the parathyroids. Both patients showed metastatic calcification in the kidneys complicated by coexisting duodenal ulcers. Treatment for the ulcer included a diet high in calcium and phosphorus and it produced in each instance an exacerbation of symptoms.

PANCREAS

Action of Insulin For many years after the experimental production of diabetes mellitus in dogs by von Mering and Minkowski in 1890 it was assumed that diabetes mellitus was purely of pancreatic origin being due to lack of the hormone later termed insulin. The effect of insulin on carbohydrate metabolism is well known: it stimulates the oxidative breakdown of glucose, has a stimulating effect on the synthesis of liver and muscle glycogen from glucose and inhibits the breakdown of liver glycogen, but how insulin acts is still little understood in spite of a vast amount of experimental work. It has not yet been possible to determine accurately how much insulin is being produced by the pancreas or to study the factors which control the activity of the islets of Langerhans. It is known that other glands are involved and that glycosuria occurs as the result of diseases of the anterior lobe of the pituitary and the adrenals. A nervous factor is also concerned and as long ago as 1855 Claude Bernard, by puncturing the floor of the fourth ventricle of the brain, induced glycosuria in the rabbit, showing that a centre in the hypothalamus was involved in the control of the blood sugar. On the other hand it is possible to maintain carbohydrate metabolism in depancreatised dogs by pancreatic grafts separated from their original nerve and blood supply.

Himsworth¹⁷ in a series of articles deals with the mechanism of diabetes mellitus and discusses the role of the liver in the control of the blood sugar level, the importance of the anterior lobe of the pituitary and the reason for the varying response of different patients to insulin. He shows that the fasting blood sugar levels of both normal and diabetic persons are maintained within definite limits. The maintenance of the blood sugar at this particular level is so important that attempts to change it by physical means are overcome by the body, indicating that the blood sugar level existing at any particular time is that which approaches nearest to the optimum for the needs of the body. This raises two questions: (i) Why should the optimal blood sugar levels vary? and (ii) How are changes in optimal levels brought about? It has been suggested that if the ability of the tissues to utilise sugar is diminished by lack of insulin or impairment of insulin action, compensation is made through an appropriate increase in the blood sugar level. In abnormal conditions the nerve centres in the brain cause a

hyperglycæmia after a few days or it is becoming worse treatment with ordinary insulin should be started with 5 to 10 units night and morning. After a few days the progress of the patient should be surveyed and the dose of insulin adjusted accordingly. This process should be repeated until the patient is stabilised on the diet he is taking. If this diet is inadequate it is raised to 150 grammes of carbohydrate and the necessary adjustment made in the insulin dose. It is important not to alter the diet and the dose simultaneously. During stabilisation it is advisable to control the process by means of blood sugar estimations but once the patient is stabilised urine tests are a satisfactory guide. When changing from ordinary to protamine zinc insulin the initial dose of protamine zinc insulin should be half the total daily dose of ordinary insulin given in one injection before breakfast. For severely ill patients ordinary insulin should not be omitted altogether until the protamine zinc insulin has begun to exert its full effect and the change should only be effected under close observation with frequent blood sugar and urine tests. When a mixture of ordinary and protamine zinc insulin is administered it is important to draw the ordinary insulin into the syringe first.

Vitamins and Blood Sugar There is some evidence that certain of the vitamins of the B group and ascorbic acid have an effect on the blood sugar level but this effect after injections of insulin appears to be variable. Martin⁴ believes that aneurine and riboflavine play a fundamental role but Demole and Silberschmidt² state that they have no effect on the blood sugar level in rabbits. Dienst⁶ studied the effects of aneurine and ascorbic acid he found that aneurine reduced the blood sugar resulting in less insulin being needed and considers that the diet of diabetics should be rich in both aneurine and ascorbic acid. Kodicek²⁷ treated twenty patients with aneurine and insulin and showed that aneurine did not affect the fasting blood sugar or increase the response of diabetics to insulin. Parr and Shipton²⁸ describe five patients in which brewers' or bakers' yeast increased the sensitivity to insulin improved the carbohydrate tolerance and diminished the amount of insulin required.

Hypoglycæmia When the blood sugar level falls below 70 milligrams per 100 millilitres symptoms of hypoglycæmia may occur namely weakness and giddiness distress or even epileptiform convulsions and coma. The prompt administration of at least 16 grammes (240 grains) of glucose or sucrose by mouth will relieve these symptoms and the dose should be repeated in half an hour if the hypoglycæmic effect of the insulin still persists. When the hypoglycæmia is severe an intravenous injection of 10 millilitres (150 minims) or more of injection of dextrose (5 per cent) should be given. Protamine zinc insulin is less likely than ordinary insulin to produce hypoglycæmia during the day and the hypoglycæmia is usually less severe than with ordinary insulin.

disadvantage of ordinary insulin is that two, or perhaps three injections daily may be necessary to maintain a satisfactory control of the blood sugar level. Nevertheless for more than ten years before the introduction of the newer types of insulin ordinary insulin permitted many thousands of diabetic to lead a comparatively normal life. Ordinary insulin is the most satisfactory preparation for use in emergencies when a prompt action is essential.

The action of protamine zinc insulin is more prolonged than that of ordinary insulin and its rate of onset is slower. In severe cases of diabetes mellitus this delaying action may cause hypoglycæmic reactions which may occur with few warning symptoms. Protamine zinc insulin is less regular than ordinary insulin in its action from day to day, probably due to variations in absorption. Because of its slow onset of action it should not be used in the treatment of hyperglycæmic coma.

Globin zinc insulin is a long acting insulin with a duration of effect and rapidity of onset intermediate between that of ordinary insulin and protamine zinc insulin. A lower blood sugar level is obtained sooner after the administration of globin zinc insulin than after protamine zinc insulin. The action of globin zinc insulin lasts about twenty four hours and with slight modifications of diet some patients with diabetes mellitus can be satisfactorily controlled by one injection a day. Unit for unit globin zinc insulin has a quantitatively greater effect than protamine zinc insulin its action being slower during the first few hours after injection than that of corresponding doses of ordinary insulin but quicker than that of protamine zinc insulin. Clinical results over a number of years have shown that globin zinc insulin although often a most useful preparation is not suitable for all patients with diabetes mellitus. The faults of globin zinc insulin are shown particularly in severe diabetes mellitus¹⁹. In large single doses it may cause late afternoon hypoglycæmia which may be countered by adjustment of the diet; moreover the effect often wanes so rapidly during the night that heavy glycosuria appears before and after breakfast.

Mixtures of protamine zinc insulin and ordinary insulin have also been employed and Colwell *et al*²⁰ reported that in these mixtures an excess of ordinary insulin is necessary to produce appreciable alterations in the effect of protamine zinc insulin. It was then shown that these mixtures contained new protamine zinc compounds with accelerated activity¹⁹. Some authorities^{1 22 23} consider that mixtures of protamine zinc insulin and ordinary insulin give a better control than globin zinc insulin and the use of two parts of protamine zinc insulin with one part of ordinary insulin seems to be most suitable.

Dosage There is no hard and fast rule for dosage and each patient must be considered individually. It is usual to study the effect of diet first starting with 100 grammes of carbohydrate daily. If on such a diet there is no appreciable fall in the degree of glycosuria or

post anæsthetic toxic symptoms acidosis and pulmonary tuberculosis but its value in these conditions is questionable

PITUITARY

The pituitary gland or hypophysis secretes a number of important hormones which stimulate or inhibit other endocrine glands. The hormones of the anterior and posterior lobes differ widely in their physiological actions and are therefore discussed separately.

ANTERIOR LOBE

Overactivity of the acidophil cells of the anterior lobe of the pituitary before the bones are fully set results in gigantism and after



FIG. 28

CUSHING'S SYNDROME
(Pituitary Basophilism)

The clinical signs include adiposity of face, neck and trunk, abnormal hair growth in females and dusky complexion with purple striae.

although severe attacks tend to occur without warning owing to the slow and gradual lowering of the blood sugar level to below the critical level. Hypoglycaemia following protamine zinc insulin may occur much later than with ordinary insulin and at unusual times for example during the night before the next day's injection or before breakfast. With small doses it may occur eight to twelve hours after the dose (i.e. before the afternoon or evening meal) and with large doses fourteen to twenty two hours after the dose (i.e. a morning dose causes hypoglycaemia in the early hours of the following morning). Hypoglycaemia following globin zinc insulin usually occurs late in the afternoon. Spontaneous hypoglycaemia can result from tumours of the islets of Langerhans when the symptoms are the same as those associated with an overdose of insulin. These tumours are however rare and palliative treatment consists in administering glucose.

Diabetic Coma Diabetic (hyperglycaemic) coma is one of the emergencies in medicine and the outcome depends very largely on the speed with which adequate treatment is instituted. The essentials of treatment are the immediate administration of full doses of insulin. If the patient is actually unconscious it is usually advisable to give the insulin intravenously. Dosage depends on the requirements of the individual patient. Lawrence and Oakley²² found that with three patients with diabetic coma 124, 142 and 196 units of insulin administered in divided doses every four hours over a period of twenty four hours removed ketosis, controlled blood sugar and restored consciousness.

Insulin in Non diabetic Conditions Insulin has an important application in the treatment of schizophrenia. Its use followed the observation of Sakel²³ that it had a quietening effect in morphine addicts deprived of opiates and when unintentional severe hypoglycaemic shocks occurred there were remarkable mental changes. The effects of insulin were tried in the treatment of schizophrenia and although other methods of treatment such as electro convulsive therapy and the use of leptazol which stimulate the central nervous system directly are also used insulin coma therapy is widely employed either alone or combined with convulsion therapy for this purpose. The treatment consists of four phases in the first of which increasing doses of insulin are administered. The second phase is characterised by shocks on each treatment day the third consists of rest periods and in the fourth small doses of insulin are given and the hypoglycaemia interrupted early.²⁰

It is known that some diabetic patients under treatment with insulin gain rapidly in weight, and insulin in small doses (5 to 10 units twenty minutes before meals)²¹ has been used in non diabetic patients to stimulate the appetite. Insulin has also been used in the treatment of

a pure form and clinical treatment with them has been disappointing. The effects produced in laboratory animals are not necessarily reproduced in humans and the relative activities of samples from different species of animals vary considerably. The action and uses of the following hormones or fractions will be considered: growth, lacticogenic, adrenocorticotrophic, thyrotrophic and gonadotrophic.

Growth Hormone. The growth factor of the anterior lobe of the pituitary stimulates the anabolic phase of protein metabolism and bears a similar relation to protein metabolism as that of insulin to carbohydrate metabolism. Overproduction of the growth hormone is

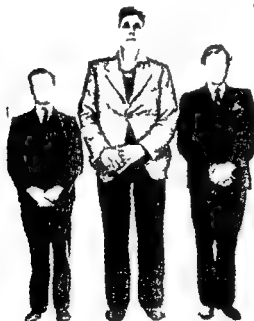


FIG. 30

GIGANTISM

The result of oversecretion of the growth hormone before the bones are fully set. The person on the right is six feet three inches.

responsible for gigantism and acromegaly. The use of a standardised growth hormone preparation in the treatment of dwarfed children has given poor results^{22, 24, 25}. Two millilitres (400 growth units Collip) are given daily by intramuscular injection and treatment with rest

ossification is complete in acromegaly. Overactivity of the basophil cells produces a condition known as Cushing's syndrome or pituitary basophilism which in many patients is accompanied by hyperplasia or adenomata of the adrenal cortex. Clinical differentiation between overactivity of the acidophil cells and of the basophil cells is difficult.

Underactivity of the anterior lobe (hypopituitarism) in children is the cause of certain types of dwarfism. In adults it results in Simmonds disease or pituitary cachexia, Frohlich's syndrome or dystrophia adiposogenitalis in which there are abnormal deposits of fat and undeveloped sexual organs as a form of hypopituitarism.

Although many physiologically active fractions have been isolated from the anterior lobe of the pituitary gland few have been obtained in

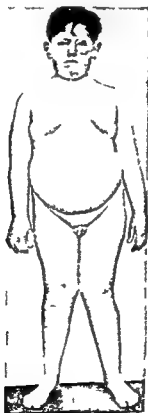


FIG. 29

FROHLICH'S SYNDROME

Characterised by general adiposity particularly of the breasts, short stght and marked knock knees

that the administration of certain cortical hormones will cause remission of symptoms in rheumatic diseases has led to the use of the adrenocorticotrophic hormone in the experimental treatment of rheumatoid arthritis and rheumatic fever³⁸

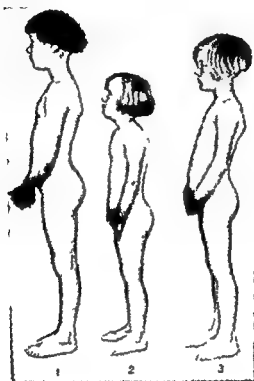


FIG. 32

DWARFISM

Caused by undersecretion of the growth hormone (1) A normal girl aged 9 (2) the patient aged 9 (3) the patient aged 7

Thyrotrophic Hormone The thyrotrophic hormone stimulates the production of thyroxine by the thyroid gland thus increasing the basal metabolic rate. Thyroid involution, which follows prolonged thyroid administration, is probably due to inhibition of the production of the thyrotrophic hormone. Bodart and Fellinger³⁹ showed that the concentration of the thyrotrophic hormone is increased in the blood of patients with hypothyroidism and Slown⁴⁰ found that the hormone does not produce a rise in the basal metabolic rate in myxoedematous patients and that a refractory state frequently occurs after about ten days' treatment due to the formation of antihormones (see page 138)

intervals has to be continued for a period of up to twelve months. It is of no value when the bones have fully set.



FIG. 31

ACROMEGALY

Oversecretion of the growth hormone after the bones are fully set causes enlargement of feet, hands and jaws with coarsening of the features. The patient is shown before the onset of acromegaly and a few years later.

Lactogenic Hormone The lactogenic hormone prolactin is also known as the luteotrophic hormone. It stimulates lactation and is secreted in increased amounts during pregnancy. Desclin³⁶ considers that the lactogenic hormone plays an important part in sex physiology, being responsible for the persistence of the corpora lutea and secretion of progesterone during pregnancy. He confirms the work of Evans *et al.*³⁷ that in addition to being a hormone of lactation, it also regulates the cycle of pregnancy and he suggests that more attention should be given to it when the luteal function requires stimulation. Clinically its use to increase lactation has proved disappointing.

Adrenocorticotrophic Hormone The function of the adrenocorticotrophic hormone (ACTH) is the stimulation of hormone production by the adrenal cortex. Theoretically, it should find application in the treatment of hypofunction of the pituitary-adrenal system and its use in Simmonds' disease has been recommended. The demonstration

but there is a risk of protein reactions in sensitive patients particularly with the less pure preparations. Chorionic gonadotrophin has a brief action, is rapidly destroyed in the body, and should be given daily to be effective. It should be given during the second half of the menstrual cycle and is usually administered intramuscularly.

Claesson *et al*⁴³ studied the action of crystalline human chorionic gonadotrophin with a potency of six to eight thousand units per milligram. Their observations on the biological effects are important and do not agree with the results of earlier observers whose investigations were carried out with relatively impure products. Many workers have stated that the chorionic hormone does not stimulate the follicles in the ovaries but Claesson and his co-workers showed that the intravenous injection of large doses of crystalline chorionic gonadotrophin (1200 units on three successive days) gave rise to increased follicular growth in the human ovary and to forced production of oestrogenic hormones. The growth of the follicles is due to a synergism between the chorionic gonadotrophin administered and the intact animal's own gonadotrophic hormones. This was confirmed by the fact that after injection hypophysectomised animals showed no growing follicles even with large doses of chorionic gonadotrophin. Moreover when chorionic gonadotrophin was combined with small doses of serum gonadotrophin there was an extensive development of the follicles. These experiments indicate that in the treatment of amenorrhoea successful results are most likely to be obtained with the use of large intravenous doses of chorionic gonadotrophin combined with serum gonadotrophin.

In the absence of genital hypoplasia Peel⁴⁴ recommends the injection of 500 to 1000 units of serum gonadotrophin every third day for three days followed by 200 to 500 units of chorionic gonadotrophin daily for three days to stimulate the normal functioning of the ovary. If menstruation follows the first course of injections further courses should be given on the ninth, eleventh, thirteenth, fifteenth, seventeenth and nineteenth days of the induced cycle. Bishop⁴⁵ although not enthusiastic about treatment with gonadotrophins states that there is some theoretical justification for the following routine: up to 2000 units of serum gonadotrophin is administered intravenously for seven days combined on the fifth, sixth and seventh days with 1000 units of chorionic gonadotrophin by intramuscular injection. The injections of chorionic gonadotrophin are then continued alone for a further four days. Rydberg and Pedersen Byrgegaard⁴⁷ found that the injection of 3000 units of serum gonadotrophin daily for five days followed by intramuscular injections of chorionic gonadotrophin 1500 units every other day for three injections produced ova and corpora lutea in the majority of patients suffering from amenorrhoea. Large doses should however always be given with discretion since there is a danger of overstimulation of the ovaries. Doses of 500 units of serum gonadotrophin daily for ten days followed by 500 units of chorionic

Gonadotrophins Clinically, the most important hormones of the anterior lobe of the pituitary gland are the gonadotrophins or gonad stimulating hormones which stimulate the ovaries in the female and the testes in the male to secrete their natural hormones. They can be extracted from the gland only with difficulty, but two standardised preparations obtained from other sources and having a somewhat similar action to the natural hormones of the anterior lobe are described in the British Pharmacopœia. They are serum gonadotrophin (Gonadotrophinum Sericum) obtained from pregnant mares serum and chorionic gonadotrophin (Gonadotrophinum Chorionicum) obtained from human pregnancy urine.

Serum gonadotrophin is considered to have a predominantly follicle stimulating action on the ovary. In the male it stimulates the germinal epithelium of the testes. Chorionic gonadotrophin formed by the chorion or lining membrane of the placenta during pregnancy has a predominantly luteinising action. In the male it stimulates the secretion of testosterone by the testes. Serum and chorionic gonadotrophins have been in use for some time but contradictory statements in the literature make it difficult to assess their clinical value. Treatment with them has not proved generally satisfactory but now that more highly refined standardised preparations are available and larger doses are employed they should have a place in sex hormone therapy when the gonads are capable of being stimulated.

Before treatment with the gonadotrophins is commenced an accurate diagnosis is essential. Ideally this should include tests for the gonadotrophin content of the urine or blood and endometrial biopsies over a period of at least three menstrual cycles. Pedersen, Bjergaard and Tonnesen⁴¹ describe techniques for estimating oestrogenic and androgenic and gonadotrophic substances in the urine and state that hormone secretion may be regarded as potentially pathological when the following values occur:

- (i) More than 30 Rat Units of gonadotrophic hormone for women before the menopause
- (ii) Less than 20 Mouse Units and more than 400 Mouse Units of oestrogenic hormone in the intermenstrual period
- (iii) More than 25 Mouse Units of oestrogenic hormone for women after the menopause
- (iv) More than 25 Capon Units of androgenic hormone

Guterman and Schroeder⁴ give details of a simplified technique for the qualitative colorimetric estimation of pregnanediol.

The method and timing of administration and the dosage of gonadotrophic hormones is also important. Serum gonadotrophin is long acting and a single injection is as effective as five divided doses given on consecutive days although daily injections have frequently been recommended. Intravenous administration may be employed

a condition in which there is an excessive excretion of urine a suitable dose is 1 millilitre (15 minims) daily administered one hour before retiring Diabetes insipidus can also be treated by nasal administration of pituitary (posterior lobe) powder Chazy and Choay⁴⁸ who treated more than a hundred patients confirm the efficacy of the nasal administration of pituitary (posterior lobe) powder and advise the use of frequent doses (four to six daily) of 0.1 to 0.2 gramme (1½ to 3 grains)

Although extracts of the posterior lobe of the pituitary gland produce valuable effects when injected there are few diseases which can be directly associated with the under or over activity of the gland Hypofunction of the gland or lesions of the middle fossa leads to diabetes insipidus and hyperfunction associated with inadequate excretion of water by the kidneys has been named diabetes tennissius by Simpson⁴⁹

Metz and Lackey⁵⁰ report on sixty seven patients with peptic ulcer treated by the intranasal insufflation of 40 milligrams (¾ grain) of pituitary (posterior lobe) powder four times daily before meals No other medication was employed and regular meals omitting only the most indigestible foods were taken three times daily This however is not a generally accepted method of treatment for peptic ulcers

ADRENALS

The adrenal or suprarenal glands are associated with the name of Addison who first observed the clinical effects of destructive disease of the glands which he considered were essential to life The adrenal glands contain two distinct types of tissue which are structurally and functionally as different as the thyroid and parathyroids It is now known that the cortex is essential to life but that the medulla may be removed without any serious effects

Adrenaline The chief hormone of the medulla adrenaline produces effects which are similar to those obtained by stimulating the sympathetic nervous system When injected intravenously it causes a rapid rise in blood pressure largely due to constriction of all the blood vessels innervated by the sympathetic nervous system together with acceleration of the heart beat dilatation of the pupil inhibition of movement of the stomach intestine and bladder and the liberation of sugar from the liver Adrenaline and related substances are therefore termed sympathomimetic compounds Adrenaline disappears rapidly from the blood stream and its action lasts for only a few minutes Although small amounts of adrenaline produce profound physiological effects there is little clinical evidence that hypofunction of the adrenal medulla exists and paroxysmal hyperfunction caused by a tumour of the glands is comparatively rare Man is much more sensitive to adrenaline than laboratory animals 0.5 to 1 milligram causing a rise in systolic blood pressure whereas this amount does not affect the blood pressure in

gonadotrophin daily for ten days have been used in the treatment of sterility due to ovulatory failure

In the male serum gonadotrophin has been used in the treatment of sterility due to defective spermatogenesis the suggested dosage being 1500 units of serum gonadotrophin intramuscularly weekly or twice weekly for four to six weeks, followed by 500 units once weekly for three or four weeks For the treatment of cryptorchidism the best age for the institution of stimulation therapy is nine to ten years and provided there is no anatomical obstruction testicular descent is frequently brought about by the administration of 500 units of chorionic gonadotrophin twice weekly For primary eunuchoidism chorionic gonadotrophin in doses of 500 units twice weekly for a period of four to six weeks sometimes produces hypertrophy of the interstitial tissue of the testis resulting in increased secretion of testosterone with concomitant improvement in the secondary sex characteristics, including growth of the genitalia and deepening of the voice Frohlich's syndrome has been treated with chorionic gonadotrophin 500 units two or three times weekly for several weeks This is sometimes effective in the male but less so in the female

POSTERIOR LOBE

Extracts of the posterior lobe of the pituitary when injected produce a gradual and prolonged rise in blood pressure and powerful contractions of the uterus They also have an antidiuretic action and increase intestinal tonicity and peristalsis Three preparations of the posterior lobe of the pituitary gland are described in the British Pharmacopœia, injection of pituitary (posterior lobe) (*Injectio Pituitarii Posterioris*) a sterile aqueous extract of the posterior lobes of mammalian pituitary bodies standardised to contain 10 units (oxytocic) in 1 millilitre injection of vasopressin (*Injectio Vasopressini*) a sterile aqueous solution containing the pressor and antidiuretic principles standardised to contain 10 units (pressor) in 1 millilitre and injection of oxytocin (*Injectio Oxytocini*) a sterile aqueous solution containing the oxytocic principle standardised to contain 10 units (oxytocic) in 1 millilitre

The two fractions oxytocic and pressor, have not been highly purified but are used instead of the whole extract when specific actions are required Injection of pituitary (posterior lobe) and injection of vasopressin are used in obstetrics to stimulate uterine contractions and to control post partum hæmorrhage After an abdominal operation they assist the intestine to recommence peristalsis and cause evacuation of the bowels Extracts of the posterior lobe should never be given before parturition if there is a possibility of an obstacle to delivery and should be administered with caution and in small doses if there are symptoms of cardiac vascular or renal disorder The dose varies from 0.2 to 1 millilitre (3 to 15 minims) intramuscularly Generally 0.2 millilitre (3 minims) is employed before delivery In diabetes insipidus

of adrenal cortical overactivity and in distinguishing between adrenocortical tumours and Cushing's syndrome (pituitary basophilism)^{55 57}

Adrenal cortical extracts were first thought to contain a single hormone which Hartman⁵⁸ called cortin. Many crystalline fractions have however been isolated and synthetic compounds prepared which indicate that the adrenal cortex exerts many different actions. The most important hormones in the cortical extracts are deoxycortone and cortisone.

Adrenal cortical extracts are particularly recommended for the treatment of adrenal crises with or without deoxycortone acetate⁵⁹. During the acute state of crisis up to 60 millilitres (2 fluid ounces) or more daily by intravenous or intramuscular injection is administered with large doses of normal saline and dextrose to restore the normal blood volume. As the patient's condition improves the dose is reduced slowly until the patient is finally maintained on injections of deoxycortone acetate every few days with the supplementary administration of sodium chloride daily. Injection of suprarenal cortex (Injectio Suprarenalis Corticis B.P.C.) will correct the deficiency in Addison's disease, increase the serum concentration of sodium and chloride and cause a drop in the serum potassium.

Deoxycortone Acetate Although injection of deoxycortone acetate does not give complete replacement therapy it is used with success in many patients with Addison's disease and it is cheaper than natural extracts. In a discussion at the Royal Society of Medicine Simpson⁶⁰ confirmed the value of deoxycortone acetate in the treatment of Addison's disease and considered that 5 milligrams is equivalent to 10 millilitres of injection of suprarenal cortex. Wilkinson⁶¹ stated that deoxycortone acetate was more satisfactory than injection of suprarenal cortex and 5 milligrams was equivalent to 20 millilitres of the natural extract. Soffer⁶² states that the daily dose should not exceed 5 milligrams ($\frac{1}{4}$ grain) unless adequate biochemical control is available.

During treatment with deoxycortone acetate an excess of sodium chloride should be avoided and adequate potassium should be administered. Gordon⁶ suggests that sodium salts should be given sparingly and should not exceed 5 grammes (75 grains) daily in addition to that already contained in the food. Oedema and hypertension are known to be affected by the salt intake and if they develop a restriction of salt is advisable. Thorn and Fiere⁶³ state that when oedema is serious increased elimination of sodium from the body can be hastened by the administration of potassium salts.

Implantation When patients have been treated for several weeks with injections of deoxycortone acetate and the approximate dosage is known the most satisfactory maintenance treatment is implantation of pellets of deoxycortone acetate under the skin. The method is economical, the need for repeated injections is eliminated and many favourable

animals Hypodermic injection causes little increase in blood pressure since absorption is restricted by local vasoconstriction

Injection of adrenaline (*Injectio Adrenalinæ B P*) is a solution of adrenaline tartrate containing adrenaline 1 in 1000 A dose of 1 millilitre (15 minims) may be used in conjunction with the parenteral or oral administration of dextrose in the treatment of hypoglycæmic attacks Adrenaline relaxes the smooth muscle of the bronchi and 0.5 to 1 millilitre (8 to 15 minims) of injection of adrenaline is usually effective in providing relief in asthma and in the treatment of anaphylactic and allergic reactions such as urticaria hay fever and serum sickness In severe asthmatic paroxysms the hypodermic needle may be left under the skin and 0.05 millilitre injected every minute (up to 10 millilitres) until the attack is controlled⁵¹ To prolong the action of adrenaline it has been administered as a suspension of adrenaline ascorbate in a mixture of wool fat wax and oil (see page 185) The average dose is 0.3 millilitre (5 minims) and its effect lasts for six to eight hours⁵² Adrenaline (1 in 100) is also administered in the form of a nasal or throat spray for prophylaxis and treatment in asthma Intra-cardiac injection of adrenaline directly into the cavity of the heart preferably the right auricle is used in the resuscitation of patients in whom the heart's action has apparently ceased through drowning poisoning or anæsthetic accidents It is contra-indicated when chloroform has been used since the adrenaline may cause ventricular fibrillation and it is not recommended in the treatment of shock⁵³

Solution of adrenaline hydrochloride (*Liquor Adrenalinæ Hydrochloridi B P*) contains adrenaline 1 in 1000 and is used locally as a hæmostatic and with local anæsthetics to localise and prolong their action and reduce hæmorrhage In dentistry the concentration used is usually adrenaline 1 in 30 000

Cortical Extracts: Adrenal cortical deficiency usually attributable to tuberculosis or atrophy of the adrenal glands, produces Addison's disease characterised by pigmentation hypotension loss of weight and anorexia with a disturbed sodium, potassium and carbohydrate metabolism Hyperfunction of the adrenal cortex or hypercorticalism produced by a tumour of the adrenal cortex or by a pituitary basophil tumour which stimulates an increased secretion of the cortical hormone leads to sexual precocity feminisation in men and virilism in women The hormones of the adrenal cortex and the gonads are closely related chemically and biologically The ovarian hormone progesterone can prolong the life of adrenalectomised animals and androgens and œstrogens can be obtained from the adrenal cortex

Urinary excretion products called 17 ketosteroids can be estimated colorimetrically⁵⁴ and the relative amounts present compared with normal levels⁵⁵ are useful in the differential diagnosis of various forms

Deoxycortone Acetate in Shock. There is experimental evidence that the symptoms of shock are partly due to cortical insufficiency but there is insufficient clinical evidence to justify the use of cortical preparations in the prevention or treatment of surgical shock^{74 75} although foetal shock in twelve infants was satisfactorily treated with normal saline and deoxycortone acetate⁷⁶

GNADS

The gonads comprising the ovaries in the female and the testes in the male secrete steroid hormones whose actions have been studied intensively in laboratory animals. The gonads, the anterior lobe of the pituitary and the adrenal glands are so interdependent that their actions should be considered together. So called male and female sex hormones are secreted by the ovaries, testes and adrenal glands and for the normal development of an individual there must be a balanced production of gonad hormones and of the pituitary gonadotrophic



FIG. 33

GYNecomastia

Enlargement of the breasts in the male may be due to excessive production of oestrogen or deficiency of androgen

results have been reported^{60 61 62 63} The hormone requirement by implantation is about 60 to 75 per cent of that required by injection but the amount of hormone absorbed depends upon many factors including the surface area and density of the pellet⁶⁴ Pellets weighing 100 milligrams (1½ grains) release approximately 0.3 milligram (½ grain) daily and implanted pellets last from six to ten months The supplementary administration of sodium chloride 2 to 5 grammes (30 to 75 grains) daily provides some measure of control since the salt can be withdrawn if there are signs of overdosage or increased when the pellets are becoming exhausted

Techniques of implantation have not been standardised Strict asepsis is of course essential Some authorities recommend a simple incision through the skin placing the compressed tablets or pellets in pockets made on either side of the incision with a pair of fine, blunt ended scissors Others use a trocar and cannula of a diameter into which the cylindrical pellets just fit the pellets being placed deeply into the underlying muscle In other cases the pellets are placed in the fat of the abdominal wall or beneath the rectus sheath The operation can be performed in from seven to ten minutes and should be carried out in the theatre Smaller doses can be inserted in the inguinal region by infiltrating a small area with procaine hydrochloride and making a stab incision through the skin The pellet is then inserted into the stab wound

Sublingual Administration Deoxycortone acetate has been administered sublingually dissolved in propylene glycol (2 milligrams in 1 millilitre)⁶⁵ The dose required is three to five times that necessary by injection the solution should be retained for at least five minutes and administered several times a day^{67 68 69 70 71}

Cutaneous Inunction Simpson⁴⁸ reports satisfactory results in selected patients with Addison's disease by rubbing the contents of one ampoule containing 20 milligrams of deoxycortone acetate in 100 millilitres of benzyl alcohol into the skin of the thigh abdomen or arm the process taking about ten to twenty minutes The dose required by this route is four times that required by intramuscular injection

Deoxycortone Acetate in Rheumatism In 1949 Lewin and Wassen⁷² described the use of deoxycortone acetate combined with ascorbic acid in the treatment of rheumatism Although this and subsequent papers reported successful results other workers failed to confirm the efficacy of the treatment Copeman *et al*⁷³ reported that it was of no significant benefit in rheumatoid arthritis and in contrast to adrenocorticotrophic hormone and cortisone produced no more improvement than could be observed to follow the injection of procaine or even normal saline

(named after Douly who with Allen introduced the vaginal smear technique) triphenylchloroethylene which has a very prolonged action on injection and D B E, a similar compound which need be given only once a week

Action of Oestrogens Natural and artificial oestrogens have essentially the same physiological action. They are responsible for the normal development of the reproductive organs and for the development of the secondary sex characteristics. Oestrogens play an important part in the complicated physiology of menstruation (see page 43). During the first half of the cycle they stimulate the regeneration of the endometrium and render it sensitive to the action of the corpus luteum hormone progesterone which acts during the second half of the cycle. Oestrogens also assist in maintaining the correct acidity of the vagina and mobilise the glycogen in the vaginal epithelium⁸⁰. A sudden withdrawal of oestrogen which normally occurs at the end of the cycle initiates menstruation. The natural hormone oestradiol is excreted in the urine mainly in the form of oestrone and oestriol both of which possess oestrogenic activity. Although oestrogens are also produced in males⁸¹, large amounts being excreted in the urine of stallions, little is known of their function in males. They appear to act synergistically with androgens⁸² but when used in the treatment of prostatic carcinoma they have either a direct action on the malignant cells or inhibit the action of androgens produced by the testes⁸³. Large doses of oestrogens inhibit the action of the anterior lobe of the pituitary and produce symptoms similar to those produced by hypophysectomy.

Methods of Administration The natural and artificial oestrogens have been administered by mouth, by injection as a solution in oil, a suspension of fine crystals or solution in water, by percutaneous application in a water miscible ointment or in a suitable organic solvent, by vaginal or rectal insertion as pessaries or suppositories and by the subcutaneous implantation of tablets or pellets.

The most effective way of administering natural oestrogens is by the injection of an ester such as oestradiol monobenzoate. Oestrone may be administered by mouth and is useful when a small dose will suffice. Percutaneous or vaginal applications are reserved for use when a high local concentration is desired and the implantation of tablets is a surgical procedure which is used only when it is desired to produce a continuous and prolonged systemic action: the complete absorption of a 100-milligram (1½ grain) tablet of stilboestrol taking about eight to nine months and a 100-milligram (1½ grain) tablet of oestradiol up to two years.

Artificial oestrogens are as potent by mouth as by injection and parenteral administration is rarely if ever necessary. They have been given as a compound tablet with hexobarbitone (16 milligrams) and

good results with a lower incidence of nausea are claimed with the use of hexoestrol phenobarbitone⁸⁴

Table XIII gives the relative doses of the oestrogens. The figures are approximate only and vary according to the condition to be treated and the response of the patient.

TABLE XIII
RELATIVE DOSES OF OESTROGENS

Oestrogen	Dose
Oestrone	2 milligrams daily by mouth
Sodium Oestrone Sulphate	3 milligrams daily by mouth
Oestradiol Dipropionate	5 milligrams every five days by injection
Oestradiol Monobenzoate	5 milligrams every three days by injection
Ethynyl-oestradiol	0.1 milligram daily by mouth
Stilboestrol	1 milligram daily by mouth
Dienoestrol	4 milligrams daily by mouth
Hexoestrol	18 milligrams daily by mouth
Dehydroxydoisynolic Acid	5 milligrams daily by mouth
Triphenylchloroethylene	100 milligrams daily by mouth
D B E	300 milligrams weekly by mouth

Toxicity The natural oestrogens are unlikely to produce any toxic effects in the doses normally employed. Artificial oestrogens sometimes produce mild nausea and vomiting which usually pass off after a few days of treatment. The toxicity of artificial oestrogens is related to the dosage employed and some workers believe that any side effects are closely associated with the oestrogenic potency of the preparation and cannot be dissociated from it⁸⁵. Although there is evidence that dienoestrol and hexoestrol are specifically less toxic than stilboestrol it seems likely that the main reason why hexoestrol only rarely produces toxic effects is due to its relatively low potency. There has been a tendency in the past to give excessive doses of artificial oestrogens and this would account for some of the untoward reactions which have been reported. Artificial oestrogens are best given late at night so that the patient may sleep through the period when toxic effects are most likely to occur. Nausea and vomiting are never encountered in pregnant women and very rarely in men.

Clinical Applications Oestrogens are of proved value in the treatment of menopausal symptoms and inflammatory states of the vagina after the menopause to prevent breast engorgement and inhibit lactation and in the treatment of gonorrhoeal vulvovaginitis in children. They are useful in certain patients with delayed puberty, secondary amenorrhoea and dysmenorrhoea. In the male they give relief in prostatic carcinoma.

Menopausal Disorders The vasomotor and psychic disturbances which are so frequently found at the menopause are due to declining oestrogen secretion by the ovaries and can, in most patients be controlled by the administration of either natural or artificial oestrogens. The menopausal woman is very sensitive to oestrogens and the dosage used should be minimal and reduced over a period of months. Bishop⁴⁵ recommends 0.25 milligram ($\frac{1}{40}$ grain) of stilboestrol daily for a fortnight and if there is no improvement in symptoms an increase in the dose to 0.5 milligram ($\frac{1}{20}$ grain) daily for a further fortnight. If necessary the dose may be further increased to 1 milligram ($\frac{1}{10}$ grain) daily during the third fortnight. When an improvement is noticed the daily dose is reduced by 0.25 milligram ($\frac{1}{40}$ grain) every fortnight if there is no return of the symptoms. Because of their effect on the vaginal mucous membranes oestrogens are of value in the treatment of the menopausal sequelae senile vaginitis and kraurosis. It is usually necessary to give high doses and an accurate diagnosis is essential to exclude early malignant disease. Treatment may consist of tablets of stilboestrol 1 to 5 milligrams ($\frac{1}{10}$ to $\frac{1}{2}$ grain) daily or injections of 5 milligrams ($\frac{1}{2}$ grain) of oestradiol monobenzoate weekly for one month. Estrone pessaries 0.1 milligram ($\frac{1}{100}$ grain) or an ointment containing natural or artificial oestrogens also help to clear up these conditions.⁴⁶

Excessive Uterine Hemorrhage It is possible to stop bleeding in patients with menorrhagia which has persisted for some time by giving high doses of stilboestrol commencing with 16 to 20 milligrams ($\frac{1}{2}$ to $\frac{3}{4}$ grain) during twenty four hours, and then 5 milligrams ($\frac{1}{2}$ grain) daily for three weeks with four injections of progesterone 10 milligrams ($\frac{1}{2}$ grain) on alternate days in the next week which will be followed by bleeding which is normal in amount. Repeated courses of 5 milligrams ($\frac{1}{2}$ grain) of stilboestrol daily for three weeks followed by progesterone may take over a difficult phase of metropathia.⁴⁷ Indeed it may be possible to achieve this with the progesterone course alone repeated every twenty eight days.

Prevention of Breast Engorgement Many workers have described the use of natural and artificial oestrogens to inhibit lactation. Lactation depends on the level of oestrogens, progesterone and gonadotrophins at parturition and also on the secretion of the lactogenic hormone by the anterior lobe of the pituitary. It is maintained by the mechanical process of suckling and when established oestrogens even in high doses will not inhibit lactation. They are however most useful in preventing painful engorgement of the breast in non nursing mothers.⁴⁸ Tablets of stilboestrol 16 milligrams ($\frac{1}{2}$ grain) on the first day, 10 milligrams ($\frac{1}{2}$ grain) on the second day and 5 milligrams ($\frac{1}{2}$ grain) daily for the rest of the first week are recommended. Prescott and Basden⁴⁹ consider that parenteral treatment with hexoestrol dipropionate is more effective than hexoestrol or stilboestrol by mouth or by injection. They found

that in most patients a single injection of 12.5 milligrams ($\frac{1}{2}$ grain) given within three days of delivery was sufficient to suppress lactation.

Gonorrhœal Vulvovaginitis in Children The object of treatment is temporarily to convert the vaginal mucosa from the child to the adult type, thereby making it resistant to the gonococcus. Brown⁹⁰ states that there is a wide variation in the dosage and the use of œstrogens is not advised after the age of ten years. Pessaries prepared with a glycerin gelatin basis have been recommended⁹¹.

œstrogens and Cancer œstrogens are in some respects chemically related to the carcinogenic hydrocarbons and their prolonged administration to highly susceptible strains of mice is sometimes followed by malignant changes⁹. When tested for carcinogenesis by painting on the skin however they are not carcinogenic. They are carcinogenic only under special conditions usually in organs concerned with reproduction. Spontaneous mammary carcinoma is known to occur in certain highly tumour sensitive strains of mice and its development is dependent on three factors (1) a genetic factor (2) a factor possibly a virus which is passed on in the milk (3) an exciting agent or œstrogen. In humans there is no evidence that œstrogens used in therapeutic doses are likely to produce cancer.

Use in Prostatic Carcinoma An interesting and rather unexpected use of œstrogens is in the treatment of prostatic carcinoma. It had been known for some time that when the testes were removed the prostate gland decreased in size. The use of œstrogens as a means of neutralising the action of testosterone or producing physiological castration was first suggested by Huggins *et al.*^{92, 94, 95}. It was soon shown that the artificial œstrogens had the same action as natural œstrogens in this condition and most of the published work on the treatment of prostatic carcinoma deals with the use of stilboestrol although hexoestrol and dienoestrol have also been employed. Huggins⁹⁴ summarises his results after five years and a review of the chemotherapy of cancer is given by Haddow⁹⁷. œstrogens are a valuable palliative measure they do not cure the condition but there seems little doubt that under treatment the foci of the disease may become quiescent, life can be prolonged and rehabilitation can be achieved⁹⁸. Surgery is still considered the procedure of choice when the original growth can be removed completely and œstrogens are best reserved as a post operative measure following radical surgery and for the relief of symptoms in terminal cases of inoperable prostatic carcinoma. œstrogens are of no value in the treatment of simple enlargement of the prostate gland.

The mode of action of the œstrogens is not clearly understood, a change in the level of the serum acid phosphatase indicates that the metabolism of the malignant cells is disorganised⁹⁹ and Fergusson and

Pigel¹⁰⁰ have shown that oestrogen therapy does bring about a reduction in the number and size of tumour cells. Oestrogens may also act through the anterior lobe of the pituitary by depressing the activity of the interstitial cells of the testes. The dosage varies considerably usually a high dosage up to 16 milligrams ($\frac{1}{2}$ grain) or more daily is recommended which frequently results in the mild gynecomastia considered desirable as an indication that treatment is proving effective.

Use in Mammary Carcinoma Some success has been achieved by the empirical treatment with oestrogens of mammary carcinoma in elderly patients but it is too early to assess their value in this condition. Binnie¹⁰¹ believes that stilboestrol combined with radiation therapy gives better results than if either is used alone. Of 168 patients reported by various workers¹⁰ forty one improved and in six of these the improvement was spectacular. The dosage varied from 1 to 16 milligrams ($\frac{1}{8}$ to $\frac{1}{2}$ grain) of stilboestrol daily but Beaumont and Dodds⁸¹ state that in responding cases 1 to 5 milligrams ($\frac{1}{8}$ to $\frac{1}{2}$ grain) daily will usually suffice to bring about relief.

PROGESTOGENS

Progesterone the naturally occurring progestogen is secreted by the corpus luteum in the second half of the menstrual cycle and by the placenta and chorionic villi during pregnancy. It converts the proliferative endometrium to a secretory phase and assists in the maintenance of pregnancy. Progesterone is excreted in the urine as an inactive water soluble compound of pregnanediol and the amount excreted is a measure of the production of progesterone by the corpus luteum and the placenta chorionic system. Excretion begins about twelve days before the onset of menstruation (a day or two after ovulation) and reaches a peak about five days later. During pregnancy the output of progesterone and therefore pregnanediol increases very considerably. Progesterone is essential for the development of the premenstrual endometrium maintenance of pregnancy desensitisation of the uterus and diminution of its power of contraction proliferation of the glandular tissue of the breast and the inhibition of ovulation during pregnancy.

Progesterone is given intramuscularly as injection of progesterone (Injectio Progesteroni B.P.) which is a sterile solution in ethyl oleate or a suitable oil and by the implantation of tablets. For oral administration ethisterone is employed usually as tablets containing 5 milligrams.

Clinical Applications The main use of progesterone and ethisterone is in the treatment of abortion. In habitual abortion treatment should be commenced as early in pregnancy as possible or even before the patient becomes pregnant. Injection of progesterone 5 to 10 milligrams ($\frac{1}{2}$ to $\frac{1}{2}$ grain) two or three times weekly is recommended.

The dose of ethisterone by mouth is approximately six times the dose of progesterone by injection the daily dosage should be divided into three or four doses and taken at intervals during the twenty four hours Six 25 milligram ($\frac{1}{2}$ grain) pellets of progesterone may be implanted as soon as a period is missed The treatment of threatened abortion is not usually so successful as the prophylactic treatment of habitual abortion and a higher dosage is usually required thus 10 to 16 milligrams ($\frac{1}{2}$ to $\frac{3}{4}$ grain) should be given until the cessation of bleeding followed by a gradual reduction in the dosage

Progesterone has been used in the treatment of menorrhagia when the condition is due to a deficiency of the progestational phase of menstruation as determined by uterine biopsy⁸⁴ It is also used in the treatment of metropathia hæmorrhagica in doses of 16 to 25 milligrams ($\frac{1}{2}$ to $\frac{3}{4}$ grain) daily for several days until the bleeding is under control when the dose is diminished Progesterone is not indicated in the treatment of dysmenorrhœa⁸⁵

ANDROGENS

The chief action of the androgens is to promote the growth of the external genital and other sexual organs they also control the secondary sex characteristics They depress the function of the anterior lobe of the pituitary and antagonise the œstrogenic hormones

The most commonly used androgen preparation is the propionic ester of testosterone administered as injection of testosterone propionate (Injectio Testosteroni Propionatis I.P.) by intramuscular injection An orally active derivative of testosterone methyltestosterone was introduced in 1935 and is given in the form of tablets usually containing 5 milligrams the dosage being three to four times that of testosterone propionate by injection

Steroid hormones such as methyltestosterone are also given sublingually by placing tablets under the tongue and allowing them to dissolve slowly¹⁰⁸ Testosterone and testosterone propionate are also applied locally in an ointment base the strengths used being from 2 to 25 milligrams per gramme Implantation tablets of 100 milligrams ($1\frac{1}{2}$ grains) are used when a prolonged sustained action is required and testosterone suppositories each containing 16 milligrams ($\frac{1}{4}$ grain) of testosterone have occasionally been recommended Although synthetic compounds having an action similar to that of the natural œstrogens have been discovered no analogous synthetic androgens have so far been prepared

Clinical Applications The principal use of androgens is for the treatment of primary hypogonadism in the male for example eunuchism and eunuchoidism The dosage varies from patient to patient usually 25 milligrams ($\frac{1}{2}$ grain) three times weekly or the oral administration of methyltestosterone in doses of 30 to 60 milligrams ($\frac{1}{2}$ to 1

grain) daily is required to produce a maximal effect. Implantation tablets of 100 milligrams ($1\frac{1}{2}$ grains) are employed when a very prolonged action is desired.



FIG. 34

EUNUCHOIDISM

Characteristics of this condition are the excessive length of limb, poor muscular development, high pitched voice and lack of facial and pubic hair.

Methyltestosterone in doses of 20 to 50 milligrams ($\frac{1}{2}$ to $\frac{3}{4}$ grain) daily has given good results in the treatment of eunuchism and eunuchoidism^{104, 105}. Androgens have also been used in the treatment of selected cases of undescended testes and they are stated to be effective in relieving the subjective symptoms of the male climacteric¹⁰⁶. Androgens are of no value in the treatment of sterility associated with aspermatogenesis and although they have been reported to give symptomatic relief in benign prostatic hypertrophy they are not a

substitute for surgery. They have given satisfactory results in a number of gynaecological complaints notably functional hæmorrhage and painful chronic mastitis and are useful in doses of 25 milligrams ($\frac{1}{2}$ grain) or more twice daily for one to three days for the suppression of lactation¹⁰⁷. Methyltestosterone in doses of 25 milligrams ($\frac{1}{2}$ grain) every four to six hours may be used twenty four hours after delivery instead of testosterone propionate to inhibit lactation. Androgen therapy in women may give rise to undesirable masculinising effects and is not recommended when oestrogens may be effective.

Use in Cancer of the Breast Androgens in the form of testosterone propionate by injection testosterone by implantation and methyltestosterone by mouth have been used in the treatment of carcinoma of the breast in young and middle aged women. A dose of 100 milligrams ($1\frac{1}{2}$ grains) of testosterone propionate by intramuscular injection three times a week for ten weeks followed by a maintenance dose of 40 to 60 milligrams ($\frac{3}{4}$ to 1 grain) of methyltestosterone by mouth for eight weeks produced palliative results and in two patients with metastases to bone there was relief of pain a feeling of well being and general symptomatic improvement¹⁰⁸. Massive doses of up to 0.2 gramme (3 grains) of testosterone propionate daily produced no satisfactory improvement in six patients treated by Herrmann and Adair¹⁰⁹ and in two patients treated by Wyatt¹¹⁰. It has been shown by several workers that androgens may be of value in the treatment of osseous metastases secondary to carcinoma of the breast¹¹¹. They have an influence on the growth processes of mammary carcinoma although clinical results have usually been disappointing but the fact that chemotherapeutic agents do have some action on the cancer cell may lead to a new approach to this problem.

ALLERGY TO HORMONES

Zondek *et al*¹¹² found that patients suffering from typical allergic symptoms were sometimes hypersensitive to their own hormones the condition of endocrine allergy being detected by the intracutaneous injection of 0.1 milligram ($\frac{1}{100}$ grain) of steroid hormone dissolved in 0.1 millilitre (14 minims) of olive oil. Tests with different hormones for the presence of hormone allergy in six patients with keratitis rosacea showed hypersensitivity to testosterone only. Desensitisation treatment with small gradually increasing doses 0.01 to 1 milligram ($\frac{1}{1000}$ to $\frac{1}{100}$ grain) in a series of twenty injections of hormone produced an improvement in the condition of every patient. Relief is obtained only when the testosterone is given in low gradually increasing doses the administration of large doses sometimes causes a severe exacerbation. When desensitisation has been achieved Zondek recommends the implantation of pellets containing 10 milligrams ($\frac{1}{4}$ grain) at intervals of three to six months to prevent recurrence of the allergic condition.

REFERENCES

- 1 COLLIP J B and ANDERSON E M *J Amer med Ass* 1935 104 965
- 2 LEATHAM J H and RAKOFF A E *J clin Endocrinol* 1948 11 267
- 3 ZONDER H *Diseases of the Endocrine Glands* Translated by C. P. Giles 2nd English Edition 1944 Arnold London.
- 4 REINHOLD J G *Amer J clin Path* 1936 6 22
- 5 ARTHUR E B *et al Endocrinology* 1943 32 210
- 6 ARTHUR E B *J Pharmacol* 1943 78 79
- 7 SIMKINS S *J clin Endocrinol* 1947 7 574
- 8 WILLIAMS R. H. *Arch intern Med* 1947 80 11
- 9 MURRAY G R *Brit med J* 1891 11 796
- 10 GROLLMAN A *Essentials of Endocrinology* 2nd Edition 1947 Lippincott London
- 11 MACBRYDE C M *J Amer med Ass* 1939 111 304
- 12 SHELLING D H *The Parathyroids in Health and Disease* 1930 Lea and Febiger Philadelphia
- 13 SCHULTZ E and CHRISTENSEN B C *Acta med Scand* 1940 123 362
- 14 VON RECKLINGHAUSEN F D *Festschrift für Rudolf Virchow* 1891 Reimer Berlin
- 15 KEATING F R Jr and COOK E A *J Amer med Ass* 1940 129 994
- 16 ROGERS H M *J Amer med Ass* 1946 130 22
- 17 HINWORTH H P *Lancet* 1939 1 65 118 171
- 18 MARBLE A *Med Clin N Amer* 1947 31 313
- 19 COLWELL A R *Arch intern Med* 1944 74 331
- 20 COLWELL A R, IZZO J L and STRYKER, W A *Arch intern Med* 1942, 69 931
- 21 MACBRYDE C M and REISS R S *J clin Endocrinol* 1944 4 469
- 22 ADLERBERG D and DOLGER, H *J Amer med Ass* 1940 128 414
- 23 MACBRYDE C M *J clin Endocrinol* 1945 5 189
- 24 MARTIN R W *Hoppe-Seyl Z* 1937 248 242
- 25 DEMOLE V and SILBERSCHMIDT R *Paris* 1937 20 292
- 26 DIENST C *Dtsch med Wsch* 1939 65 710
- 27 KODICEK E *Lancet* 1942 1 501
- 28 PARR, L J A and SHIPTON E A *Med J Aust* 1947 1 365
- 29 SAKEL, M *Pharmacological Shock Treatment of Schizophrenia* Translated by J WORTH 1939 Nervous and Mental Disease Publishing Co. Washington
- 30 JESSNER L and RYAN A G *Shock Treatment in Psychiatry* 1942 Heinemann London
- 31 METZ R B *Ann intern Med* 1932 6 743
- 32 LAWRENCE R D and OAKLEY W *Brit med J* 1948 11 310
- 33 SHELTON E A, CAVANAUGH L A and FLANN H M *Amer J Dis Child* 1936 52 100
- 34 SCHAFER R L *Endocrinol* 1936 20 64
- 35 SCHAFER R L and STRICKRODT F L *Endocrinology* 1940 26 599
- 36 DESCLIN L *Brux med* 1948 28 1107
- 37 EVANS H M *et al Endocrinol* 1941 28 933
- 38 HENCH P S, KENDALL, E C, SLOCUM C. H and POLLEY H P *Proc May Clin* 1949 24 181
- 39 BODART F and FELLINGER A *Wien klin Wsch* 1936 49 1786
- 40 SCOWEN E F *Lancet* 1937 11 799
- 41 PEDERSEN BJERGAARD K and TØNNESEN A *Acta Endocrinol* 1948 1 38
- 42 GUTERMAN H S and SCHROEDER M S *J Lab Clin Med* 1948 33 306
- 43 CLAESON L *et al Acta Endocrinol* 1948 1 1
- 44 PELL, J H *Textbook of Gynecology* 2nd Edition, 1946 Heinemann London
- 45 BISHOP P M F *Gynecological Endocrinology for the Practitioner* 1946 Livingstone Edinburgh
- 46 BISHOP P M F *Practitioner* 1946 156 465
- 47 RYDBERG E and PEDERSEN BJERGAARD K *J Amer med Ass* 1943 121 1117
- 48 CHOAY A and CHOAY L *P med* 1946 54 500
- 49 SIMPSON S L *Major Endocrine Disorders* 2nd Edition 1943 Oxford University Press London
- 50 METZ M H and LACEY R W *Amer J Surg* 1940 71 27

substitute for surgery. They have given satisfactory results in a number of gynaecological complaints notably functional hæmorrhage and painful chronic mastitis and are useful in doses of 25 milligrams ($\frac{1}{2}$ grain) or more twice daily for one to three days for the suppression of lactation¹⁰⁷. Methyltestosterone in doses of 25 milligrams ($\frac{1}{2}$ grain) every four to six hours may be used twenty four hours after delivery instead of testosterone propionate to inhibit lactation. Androgen therapy in women may give rise to undesirable masculinising effects and is not recommended when oestrogens may be effective.

Use in Cancer of the Breast Androgens in the form of testosterone propionate by injection, testosterone by implantation and methyltestosterone by mouth have been used in the treatment of carcinoma of the breast in young and middle aged women. A dose of 100 milligrams (1½ grains) of testosterone propionate by intramuscular injection three times a week for ten weeks followed by a maintenance dose of 40 to 60 milligrams ($\frac{3}{4}$ to 1 grain) of methyltestosterone by mouth for eight weeks produced palliative results and in two patients with metastases to bone there was relief of pain, a feeling of well being and general symptomatic improvement¹⁰⁸. Massive doses of up to 0.2 gramme (3 grains) of testosterone propionate daily produced no satisfactory improvement in six patients treated by Herrmann and Adair¹⁰⁹ and in two patients treated by Wyatt¹¹⁰. It has been shown by several workers that androgens may be of value in the treatment of osseous metastases secondary to carcinoma of the breast¹¹¹. They have an influence on the growth processes of mammary carcinoma although clinical results have usually been disappointing but the fact that chemotherapeutic agents do have some action on the cancer cell may lead to a new approach to this problem.

ALLERGY TO HORMONES

Zondek *et al*¹¹² found that patients suffering from typical allergic symptoms were sometimes hypersensitive to their own hormones, the condition of endocrine allergy being detected by the intracutaneous injection of 0.1 milligram ($\frac{1}{100}$ grain) of steroid hormone dissolved in 0.1 millilitre (12 minims) of olive oil. Tests with different hormones for the presence of hormone allergy in six patients with keratitis rosacea showed hypersensitivity to testosterone only. Desensitisation treatment with small, gradually increasing doses 0.01 to 1 milligram ($\frac{1}{100}$ to $\frac{1}{10}$ grain) in a series of twenty injections of hormone produced an improvement in the condition of every patient. Relief is obtained only when the testosterone is given in low gradually increasing doses; the administration of large doses sometimes causes a severe exacerbation. When desensitisation has been achieved, Zondek recommends the implantation of pellets containing 10 milligrams ($\frac{1}{5}$ grain) at intervals of three to six months to prevent recurrence of the allergic condition.

- 102 *Proc R. Soc Med* 1944 37 731
- 103 SPENCE A. W. *Brit med J* 1942 ii 668
- 104 FOSS G. L. *Brit med J* 1933 ii 11
- 105 VEST S. A. and BARELARE B. Jr. *J Amer med Ass* 1941 117 1421
- 106 WERNER A. A. *J Amer med Ass* 1946 132 188
- 107 KURZROB R. and O'CONNELL, C. P. *Endocrinology* 1938 23 476
- 108 ADAIR F. E. *Med Clin N Amer* 1948 32 18
- 109 HERRMANN J. B. and ADAIR F. E. *J In Endocrinol* 1946 6 769
- 110 WYATT J. *J Obstet Gynec* 1945 52 174
- 111 HERRMANN J. B. ADAIR F. E. and WOODARD H. Q. *Surgery* 1947 22 101
- 112 ZONDER, H. BROMBERG Y. M. and LANDAU J. *Nature Lond* 1947 159 171

- 51 COLE L *Practitioner* 1942 148 204
- 52 KENNEDY A *Lancet* 1941 ii 279
- 53 MINOT A B and BLALOCK A *Ann Surg* 1940 112 557
- 54 CALLOW N H CALLOW R K and EMNENS C W *Biochem J* 1938 32 1312
- 55 HAMBURGER C *Acta Endocrinol* 1948 1 19
- 56 BROSTER L K *Brit med J* 1940 i 425
- 57 BROSTER L R *Practitioner* 1947 158 307
- 58 HARTMAN F A LEWIS L and TOBY C G *Science* 1937 86 128
- 59 SOFFER L J Diseases of the Adrenals 2nd Edition 1948 Kimpton London
- 60 SIMPSON M L *Proc R Soc Med* 1939 32 685
- 61 WILKINSON J F *Proc R Soc Med* 1939 32 689
- 62 GORDON E S *J Amer med Ass* 1940 114 2549
- 63 THORN G W and FIROR W M *J Amer med Ass* 1940 114 2517
- 64 SOFFER L J ENGEL J F and OPPENHEIMER B S *J Amer med Ass* 1940 115 1860
- 65 THORN G W HOWARD R P and EMERSON K Jr *J clin Invest* 1939 18 449
- 66 McCULLAGH F P LEWIS L J and SHIVELY F L *J clin Endocrinol* 1943 3 493
- 67 WILSON A *Lancet* 1942 i 762
- 68 ANDERSON F HAYMAKER W and HENDERSON M *J Amer med Ass* 1940 115 2167
- 69 TURNOFF D and ROWNTREE L G *J Amer med Ass* 1941 116 2010
- 70 THORN G W DORRANCE S S and DAY E *Ann intern Med* 1942 16 1053
- 71 DUNLOP D M *Brit med J* 1943 i 557
- 72 LEWIN E and WASSEN E *Lancet* 1949 ii 993
- 73 COPEMAN W S C et al *Lancet* 1950 i 830
- 74 INGLE D J *Ann Rev Physiol* 1945 7 527
- 75 KEATING F R Jr POWER M III and RYNEARSON F H *Anesth and Analges* 1942 21 207
- 76 KEATING F R Jr et al *J Obstet Gynec* 1942 49 573
- 77 FRIED I H and HAIR Q *J clin Endocrinol* 1943 3 512
- 78 BISHOP P M F KENNEDY G C and WYNN WILLIAMS G *Lancet* 1948 ii 764
- 79 PRESCOTT F and BASDEN M *Brit med J* 1944 ii 428
- 80 RAKOFF A E GEO L G and GOLDSTEIN L *Amer J Obstet Gynec* 1944 47 467
- 81 DINGEMANSE E LAQUEUR E and MUEHLBOCK O *Nature Lond* 1938 141 927
- 82 KORENCHIEVSKY V and DENNISON M *Biochem J* 1937 31 862
- 83 SCHERKEN J R BURNS E L and KAHLE P J *J Urol* 1942 48 99
- 84 LANE F E *West J Surg* 1944 52 313
- 85 BEAUMONT G E and DODDS E C Recent Advances in Medicine 12th Edition 1947 Churchill London
- 86 DODDS E C *Practitioner* 1944 152 129
- 87 BISHOP P M F *Practitioner* 1948 161 211
- 88 ABARBANEL A R and GOODFRIEND M J *Amer J Obstet Gynec* 1940 40 1037
- 89 NOVAK E *J clin Endocrinol* 1943 3 274
- 90 BROWN W E *Amer J Dis Child* 1942 64 221
- 91 CUSHNY A R Pharmacology and Therapeutics 13th Edition 1947 Revised by A GROLLMAN and D SLAUGHTER Churchill London
- 92 GARDNER W V et al *J Amer med Ass* 1936 107 656
- 93 HUGGIN C and HODGES C V *Cancer Research* 1941 1 293
- 94 HUGGINS C STEVENS R E Jr and HODGES C V *Arch Surg* 1941 43 209
- 95 HUGGIN C SCOTT W W and HODGES C V *J Urol* 1941 46 997
- 96 HUGGINS C *J Amer med Ass* 1946 131 576
- 97 HADDOW A *Brit med Bull* 1947 4 417
- 98 FERGUSON J D *Lancet* 1946 ii 551
- 99 KING A J and DELORY G E *Post Grad med J* 1948 24 299
- 100 FERGUSON J D and PAGEL W *Brit J Surg* 1945 33 122
- 101 BINNIE G G *Brit J Radiol NS* 1944 17 42

Injectio Insulini B P (Injection of Insulin Insulinum Insulin) is also called ordinary regular soluble or unmodified insulin. It consists of a sterile solution of the specific antidiabetic principle of mammalian pancreas in water of pH 3 to 4 containing an antiseptic to inhibit the development of any organism introduced during use.

The active principle may be prepared from finely divided fresh or frozen pancreas by extraction with acidified aqueous alcohol followed by filtration. The filtrate which contains the insulin is freed from unwanted proteins by concentrating to low bulk and adding more alcohol. Much of the unwanted protein material is precipitated when the concentration of ethyl alcohol is between 60 and 70 per cent and filtered off. The concentration of alcohol is then increased to 90 per cent and the precipitated material collected and dissolved in water. From this solution the active material is obtained by adjusting the reaction to the iso-electric point which is between pH 5 and pH 6 and filtering. Instead of separating the active material from aqueous solution by the iso-electric point method it may be obtained by adding trinitrophenol and precipitating as a complex. The complex is decomposed by dissolving in acidified aqueous alcohol and pouring the solution into an excess of acetone. The precipitate obtained by either method is dried and powdered and the powder is dissolved in water of pH 3 to 4 to which the required antiseptic is added. It is sterilised by filtration, assayed biologically and adjusted to contain 20, 40 or 80 units in each millilitre. It is packed in sterile glass multiple dose containers which are sealed with rubber caps or by other methods to allow the withdrawal of doses on different occasions. The labels on the containers should state the strength, the date of manufacture and the date after which the injection should not be used. Injection of insulin is a clear colourless liquid and should not be used if it develops a turbidity. It should be stored at a temperature just above its freezing point and not exposed to temperatures above 20°. Under these conditions it should retain its potency for at least two years if the reaction of the solution remains between pH 3 and 4. Injection of insulin is usually given by subcutaneous injection but may be given intravenously in certain circumstances. The dose is determined by the physician in accordance with the needs of the patient. Unless otherwise stated injection of insulin containing 20 units in each millilitre should be dispensed.

Injectio Insulini Protaminati cum Zinc B P (Injection of Protamine Zinc Insulin Protamine Zinc Insulin) is a sterile suspension of a combination of insulin and a suitable protamine such as that obtained from the milt of the salmon trout together with a small proportion of zinc chloride. The suspension also contains glycerin, sufficient sodium phosphate to maintain the reaction between pH 6.9 and 7.3 and an antiseptic to inhibit the development of any organism introduced during

CHAPTER VII

PHARMACY

PREPARATIONS of hormones may be divided for pharmaceutical purposes into two groups. The first group comprises those in which the active principle is of unknown chemical constitution and includes the proteins of the parathyroid, pancreas and pituitary glands and the gonadotrophins. The second group comprises those in which the active principle is of known chemical constitution and includes thyroxine, adrenaline and the steroid hormones of the adrenal cortex and gonads together with their artificial analogues. With the first group of preparations the chief concern of the pharmacist is with the preservation of the potency and storage since they are usually supplied by the manufacturers ready for use. With the second group there is ample scope for the application of pharmaceutical skill in presenting the active principles in the most suitable form for the particular purpose required and in ensuring maximum activity and stability.

PREPARATIONS OF HORMONES OF UNKNOWN CONSTITUTION

Parathyroids. The parathyroid hormone is ineffective by mouth and is usually administered intramuscularly as parathyroid injection.

Parathyroid injection is official in the U.S.P. XIV and consists of a sterile aqueous solution prepared from the fresh parathyroid glands of animals used for food by man. The glands are removed immediately the animals are killed and extracted at once or kept frozen until extracted. They are freed from fat and connective tissue, ground and extracted. The extract is sterilised preferably by filtration and adjusted to a potency of not less than 100 U.S.P. Parathyroid Units per millilitre. The injection should be packed in hermetically sealed containers preferably containing a single dose, and stored at a temperature not greater than 15°. It may become slightly turbid or form a slight precipitate. An expiry date after which it is to be regarded as unsuitable for use is not given, but most manufacturers allow eighteen months. The average dose is stated as 25 U.S.P. Parathyroid Units by intramuscular injection.

A parathyroid extract prepared in a somewhat similar manner was described in the British Pharmaceutical Codex 1934 but has been omitted from the British Pharmaceutical Codex 1949.

Pancreas. The hormone of the pancreas, insulin, resembles the parathyroid hormone in that it is destroyed in the alimentary tract and cannot be given by mouth. In the treatment of diabetes mellitus it is administered by injection in one of the forms described below. It is also used in the treatment of certain conditions by local application.

Injectio Insulini B P (Injection of Insulin Insulinum Insulin) is also called ordinary regular soluble or unmodified insulin. It consists of a sterile solution of the specific antidiabetic principle of mammalian pancreas in water of pH 3 to 4 containing an antiseptic to inhibit the development of any organism introduced during use.

The active principle may be prepared from finely divided fresh or frozen pancreas by extraction with acidified aqueous alcohol followed by filtration. The filtrate which contains the insulin is freed from unwanted proteins by concentrating to low bulk and adding more alcohol. Much of the unwanted protein material is precipitated when the concentration of ethyl alcohol is between 60 and 70 per cent. and filtered off. The concentration of alcohol is then increased to 95 per cent. and the precipitated material collected and dissolved in water. From this solution the active material is obtained by adjusting the reaction to the iso electric point, which is between pH 5 and pH 6 and filtering. Instead of separating the active material from aqueous solution by the iso-electric point method it may be obtained by adding trinitrophenol and precipitating as a complex. The complex is decomposed by dissolving in acidified aqueous alcohol and pouring the solution into an excess of acetone. The precipitate obtained by either method is dried and powdered and the powder is dissolved in water of pH 3 to 4 to which the required antiseptic is added. It is sterilised by filtration, assayed biologically and adjusted to contain 20, 40 or 80 units in each millilitre. It is packed in sterile glass multiple dose containers which are sealed with rubber caps or by other methods to allow the withdrawal of doses on different occasions. The labels on the containers should state the strength, the date of manufacture and the date after which the injection should not be used. Injection of insulin is a clear colourless liquid and should not be used if it develops a turbidity. It should be stored at a temperature just above its freezing point and not exposed to temperatures above 20°. Under these conditions it should retain its potency for at least two years if the reaction of the solution remains between pH 3 and 4. Injection of insulin is usually given by subcutaneous injection but may be given intravenously in certain circumstances. The dose is determined by the physician in accordance with the needs of the patient. Unless otherwise stated injection of insulin containing 20 units in each millilitre should be dispensed.

Injectio Insulini Protaminati cum Zinc B P (Injection of Protamine Zinc Insulin Protamine Zinc Insulin) is a sterile suspension of a combination of insulin and a suitable protamine such as that obtained from the milt of the salmon trout together with a small proportion of zinc chloride. The suspension also contains glycerin, sufficient sodium phosphate to maintain the reaction between pH 6.9 and 7.3 and an antiseptic to inhibit the development of any organism introduced during

use. It is an almost colourless turbid liquid containing 40 or 80 units in each millilitre and should be packed, labelled and stored in the manner described for ordinary insulin except that the label should also direct that the container be well shaken before use. Injection of protamine zinc insulin is always given by subcutaneous injection. The dose is determined by the physician in accordance with the need of the patient. Unless otherwise stated, injection of protamine zinc insulin containing 40 units in each millilitre should be dispensed.

Injectio Insulini Globini cum Zinco (Injection of Globin Zinc Insulin, Globin Zinc Insulin) is a sterile aqueous solution of a combination of insulin and the protein globin obtained from haemoglobin together with a small proportion of zinc chloride. It also contains glycerin and an antiseptic to inhibit the development of any organism introduced during use and the reaction is adjusted to about pH 3.5. It is sterilised by filtration. Injection of globin zinc insulin is an almost clear, colourless liquid. It has approximately the same stability as ordinary insulin and the same conditions of packing, labelling and storage should be applied.

Preservation of Insulin There has been considerable controversy about the desirability of adding antiseptics to aqueous preparations of insulin. Leyton and Poulton³ and Bennett² doubt the necessity of any addition and Sandor³ states that the compulsory addition is injurious. On the other hand, Hartley⁴ showed that whereas preparations of pH 3 to 4 are germicidal, a slight increase in alkalinity nullifies the germicidal action and if no antiseptic is present converts the preparation into a culture medium.

Identification of Insulin Packs To facilitate recognition of the various types of insulin, the colour scheme for labels and packages described in Table XIV has been adopted by the manufacturers in Great Britain.

TABLE XIV
IDENTIFICATION COLOURS FOR INSULIN PACKS

Type and Strength	Colour of Label and Package
<i>Injection of Insulin</i> 20 units per millilitre 40 units per millilitre 80 units per millilitre	Buff Blue Green
<i>Injection of Globin Zinc Insulin</i> 40 units per millilitre 80 units per millilitre	Blue and brown Green and brown
<i>Injection of Protamine Zinc Insulin</i> 40 units per millilitre 80 units per millilitre	Blue and pink Green and pink

Insulin Tablets Insulin may be made into tablets using an inert substance such as lactose or dextrose as diluent. The tablets should be issued in sealed sterile containers labelled with the number of units in each tablet and should yield a clear sterile solution when dissolved in water for injection.

Insulin for Local Application Insulin has been used locally in the treatment of varicose ulcers either as a wet dressing or as an ointment in wool fat or ointment of wool alcohols.

Anterior Lobe of the Pituitary Preparations of those hormones of the anterior lobe which are available commercially consist mainly of mixtures of the hormones in which one particular effect predominates.

Growth Hormone This is available in concentrated forms usually containing traces of the thyrotrophic and gonadotrophic hormones. The preparations are standardised in rat growth units and are usually supplied in multiple dose containers which should be stored in a refrigerator. They should be used within six months of manufacture.

Gonadotrophic Hormones Several preparations are available containing what is called the gonadotrophic hormone either alone or in various combinations and standardised in a variety of units. There is little information concerning the stability of the gonadotrophic hormones and they should be kept in a cool place.

Lactogenic Hormone (Prolactin Luteotrophic Hormone) This hormone is stable under ordinary conditions and may be stored at room temperature. Lyons⁵ reports that a 1 per cent solution at pH 7.6 may be heated at 100° for twenty minutes without loss of potency.

Thyrotrophic Hormone Preparations of this hormone either alone or with gonadotrophic hormones are issued in multiple dose containers.

Adrenocorticotrophic Hormone (ACTH) is supplied as a solution or as a sterile powder to be dissolved before use in an aqueous solvent containing an antiseptic. It is stable to heat and may be stored at ordinary temperatures. A method of preparation is described by Reiss and Halkerston⁶. The best source is pig pituitary, 1 kilogram yielding enough for 600 to 800 25 milligram ampoules. A crude preparation is first obtained by extracting the minced fresh or frozen glands three or four times with acetone and hydrochloric acid, diluting the solution thus obtained with acetone and filtering. A further yield is obtained from the filtrate by treatment with trinitrophenol followed by ammonia and a large excess of acetone. The combined precipitates constitute the crude preparation which is free from the gonadotrophic growth and thyrotrophic hormones but contaminated with a small amount of the

lactogenic hormone and with the hormones of the posterior lobe. The lactogenic hormone is removed by precipitation with sodium chloride at pH 3. The posterior lobe hormones are removed by extraction with ammoniacal methyl alcohol in which they are more soluble than adrenocorticotrophic hormone. The described method gives three apparently distinct fractions of the adrenocorticotrophic hormone all nearly completely free from oxytocic activity. The fractions are washed with acetone, dried, assayed and packed into ampoules. The powders may be heated at 180° to 200° *in vacuo* without loss of activity, but with loss of solubility; this effect may be useful in reducing the very rapid rate of excretion of the hormone. Concentrated solutions may be filtered through a Seitz filter or irradiated by a 30 watt ultraviolet lamp without loss of activity.

Pituitary like Gonadotrophins Two gonadotrophic hormones from other sources have been described as pituitary like because of their close resemblance in action to the follicle stimulating and luteinising hormones. They are chorionic gonadotrophin from human pregnancy urine and serum gonadotrophin from the serum of pregnant mares.

Gonadotrophinum Chorionicum B.P. (Chorionic Gonadotrophin) is obtained from the urine of pregnant women. The urine is freed from inert material, alcohol is added and the reaction is adjusted to pH 5. The precipitated material is collected, washed and dried. It is assayed biologically and diluted if necessary with a neutral powder to a potency equal to that of the standard preparation. It is a white or fawn coloured powder, soluble in water, and provided it is stored in hermetically sealed containers at a temperature not higher than 20° retains its potency for twelve months. Containers must be labelled with the total number of units and the date of manufacture. Injection of chorionic gonadotrophin (*Injectio Gonadotrophini Chorionici B.P.*) is prepared immediately before use by dissolving the contents of a sealed container in the required amount of water for injection containing 0.5 per cent of phenol. The dose of chorionic gonadotrophin is 100 to 500 units by intramuscular injection.

Gonadotrophinum Sericum B.P. (Serum Gonadotrophin) is prepared from the blood taken from pregnant mares between the sixtieth and seventy-fifth days of pregnancy in a manner similar to that used in preparing chorionic gonadotrophin. It is assayed biologically and adjusted to a potency equal to that of the standard preparation. It is a white powder, soluble in water and is subject to the same storage and labelling requirements as chorionic gonadotrophin. Injection of serum gonadotrophin (*Injectio Gonadotrophini Serici B.P.*) is prepared immediately before use by dissolving the contents of a sealed container in the

required amount of water for injection containing 0.5 per cent of phenol. The dose of serum gonadotrophin is 200 to 1000 units by intramuscular injection.

Mixtures of Gonadotrophic Hormones A mixture of chorionic gonadotrophin and an extract of the anterior lobe containing the follicle stimulating hormone is available as a dry powder and in liquid form. It should be stored in a cool place. The powder retains its activity for several years but the liquid preparation should be used within six months of the date of manufacture.

Posterior Lobe of the Pituitary Extracts of the posterior lobe contain the oxytocic, pressor and antidiuretic principles. The oxytocic principle can be almost completely separated from the pressor and antidiuretic principles and all three preparations (total extract, oxytocic extract and pressor and antidiuretic extract) are used as pharmacological or therapeutic tools rather than for replacement therapy.

Injectio Pituitarii Posterioris B.P. (Injection of Pituitary (Posterior Lobe) Extractum Pituitarii Liquidum Pituitary (Posterior Lobe) Extract Pituitary Extract Posterior Pituitary Injection) is the complete aqueous extract of the posterior lobes of mammalian pituitary glands. The glands are removed from the animals as soon as possible after death and immediately frozen. The posterior lobes are separated, subdivided and either used at once or dehydrated and powdered. The extract is prepared by immersing the frozen material or the dry powder in distilled water acidified to pH 3 to 4 with acetic acid and sufficiently hot to coagulate proteins and destroy autolytic enzymes. After filtration the extract is assayed and if necessary diluted to a potency of 10 units (oxytocic) per millilitre and the reaction again adjusted to pH 3 to 4. The extract is sterilised either by filtration before being placed in glass containers or by heating in an autoclave after sealing in the containers. The containers should be sealed glass ampoules or multiple dose containers. When the injection is sterilised by filtration or when it is supplied in multiple dose containers it must contain a bacteriostatic. Injection of pituitary (posterior lobe) is a clear, colourless liquid with a faint odour. Provided its reaction remains between pH 3 and 4 and it is stored just above its freezing point it should retain its activity for eighteen months. It should be labelled with the number of units (oxytocic) in a millilitre, the date of manufacture and the date after which it is not to be used and may also bear the number of units (antidiuretic) and units (pressor) in each millilitre. The dose of injection of pituitary (posterior lobe) is 0.2 to 0.5 millilitre (3 to 8 minims) by subcutaneous or intramuscular injection.

Injectio Oxytocina B.P. (Injection of Oxytocin Oxytocin) is a sterile aqueous solution of the oxytocic fraction of an extract of the posterior

lobe of mammalian pituitary glands adjusted to a potency of 10 units (oxytocic) in a millilitre. Any pressor activity must not be greater than that corresponding to 0.5 unit (pressor) in a millilitre. The methods of sterilising, packing, labelling and storing are similar to those for injection of pituitary (posterior lobe). The dose of injection of oxytocin is 0.5 to 1 millilitre (8 to 15 minims) equivalent to 5 to 10 units (oxytocic) by subcutaneous or intramuscular injection.

Injectio Vasopressini B.P. (Injection of Vasopressin. Vasopressin) is a sterile solution containing the pressor and antidiuretic fraction of an extract of the posterior lobe of mammalian pituitary glands adjusted to a potency of 10 units (pressor) in a millilitre. Any oxytocic activity present should not be greater than that corresponding to 1 unit (oxytocic) per millilitre. The methods of sterilising, packing, labelling and storing are similar to those for injection of pituitary (posterior lobe). The dose of injection of vasopressin is 0.5 to 1.5 millilitres (8 to 25 minims), equivalent to 5 to 15 units (pressor) by subcutaneous or intramuscular injection.

For a prolonged antidiuretic action the most suitable preparation consists of an oily suspension of the tannate of the pressor principle. A solution of the pressor principle is treated with tannic acid and the resulting precipitate suspended in arachis oil. The suspension is usually adjusted to a potency of 5 units (pressor) per millilitre and is stable at room temperature.⁷

Pituitary (Posterior Lobe) Emulsion has also been used to obtain a prolonged antidiuretic action but it has several disadvantages when compared with the tannate suspension chiefly the formation of paraffinoma and is now seldom used.^{7, 8} It may be prepared according to the following formula:

Injection of Pituitary (Posterior Lobe)	40 ml
Lactic Acid	0.4 ml
Wool Fat	0.5 g
White Beeswax	0.2 g
Chloroxylenol	0.01 g
Oil c.O.I.	a sufficient quantity

Mix the injection of pituitary (posterior lobe) and the lactic acid and concentrate *in vacuo* over sulphuric acid to 4 millilitres. Mix the remaining ingredients using sufficient olive oil to produce 6 millilitres in a wide mouthed stoppered bottle and heat at 150° for one hour. Allow the mixture to cool to 40° and gradually add the pituitary concentrate shaking between each addition to emulsify. On cooling the emulsion solidifies and must be warmed to about 40° before administration. To avoid destruction of the active principles care must be taken not to overheat the preparation.

Pituitary (Posterior Lobe) Powder is used as a snuff. It is prepared from mammalian pituitary bodies by immersion in several changes of acetone, drying, powdering and assaying. The powder is usually diluted with lactose. It occurs as a yellowish or greyish powder with a characteristic odour. A water miscible nasal jelly has also been used as a means of administering the antidiuretic principle of the posterior lobe.

A suitable preparation may be made by dissolving 0.5 to 1 per cent of sterculia or about 3 per cent of tragacanth in a solution of vasopressin containing 10 units per millilitre and adding a preservative such as 0.5 per cent of chlorbutol. It should be supplied in collapsible metal tubes fitted with nozzles suitable for nasal use.

PREPARATIONS OF HORMONES OF KNOWN CONSTITUTION

Thyroid Although thyroxine, the hormone of the thyroid gland, is available as a pure crystalline substance it is usually administered as the dried gland.

Thyroideum B.P. (Thyroid, *Thyroideum Siccum*, Dry Thyroid, Thyroid Extract, Thyroid Gland) is prepared from the thyroid glands of oxen, sheep and pigs by drying, powdering, defatting and again drying. It is assayed and diluted with lactose to contain 0.09 to 0.11 per cent of iodine in combination as thyroxine. It occurs as a buff coloured powder and should be stored in well closed containers in a cool place. It is almost invariably administered as tablets (*Tabellæ Thyroides B.P.*) if the strength of the tablet is not prescribed. 1 grain tablets should be dispensed. The dose of thyroid is 30 to 120 milligrams ($\frac{1}{2}$ to 2 grains).

A glycerin extract of thyroid was described in the British Pharmacopœia 1898 under the title *Liquor Thyroides* and had a considerable use although it has now fallen out of favour. It is occasionally used to make a thyroid ointment by incorporation with ointment of wool alcohols using 1 millilitre to each gramme of ointment of wool alcohols.

Thyroxine is usually administered as the sodium salt which was included in the British Pharmacopœia 1932 under the name thyroxine sodium (*Thyroxinsodium*) but was omitted from the British Pharmacopœia 1948. The substance described consisted of the sodium salt of racemic thyroxine which was much less active weight for weight than the naturally occurring L-thyroxine. L-Thyroxine is now available commercially as the sodium salt and is used as tablets containing 0.05 and 0.1 milligram. It is also given by injection as a solution of the sodium salt in water for injection. The British Pharmaceutical Codex 1934 directed that solutions for injection should be prepared immediately before use by dissolving in water for injection. New and Non-official Remedies 1947 states that a solution of freshly prepared thyroxine sodium may be sterilised by immersion in boiling water. Thyroxine ointment is prepared by dissolving 2 milligrams of thyroxine sodium in 1 millilitre of water and incorporating the solution in 1 gramme of ointment of wool alcohols.

Antithyroid Substances used in the treatment of hyperthyroidism include thiouracil, methylthiouracil and propylthiouracil. They are usually administered as tablets.

Adrenals : The hormone adrenaline secreted by the medulla is used as a pharmacological and therapeutic tool whereas the steroid hormones of the cortex are used for replacement therapy. Since the cortical hormones are similar to the natural sex hormones they are treated together under the section entitled Steroid Hormones.

Adrenalina B P (Adrenaline Adrenalinum Epinephrine) is prepared from an acid extract of adrenal glands or by synthesis. It is a colourless or pale buff crystalline powder sparingly soluble in water. Adrenaline is stable if stored in dark glass containers; neutral or alkaline solutions rapidly become red on exposure to the air. For local application it is used as solution of adrenaline hydrochloride and for parenteral administration as injection of adrenaline.

Liquor Adrenalinae Hydrochloridi B P (Solution of Adrenaline Hydrochloride Epinephrine Solution) is an aqueous solution of adrenaline hydrochloride containing chlorbutol sodium chloride sodium metabisulphite and adrenaline 1 in 1000. It should be stored in small well filled containers in a cool place and protected from light. It is not a sterile preparation and is intended for topical use either alone or added to other preparations.

Injectio Adrenalina B P (Injection of Adrenaline Injection of Adrenaline Tartrate) is a sterile aqueous solution of adrenaline tartrate containing sodium chloride sodium metabisulphite and adrenaline 1 in 1000. The tartrate is more suitable than the hydrochloride for the preparation of an injection because provided the solution contains 0.1 per cent of sodium metabisulphite and the reaction is maintained at pH 3.6 it can be sterilised by heating in an autoclave⁹. In preparing the injection the pharmacopœial method should be strictly followed since if the tartaric acid solution is too dilute the adrenaline dissolves with difficulty and if the adrenaline is added before the sodium metabisulphite it may be oxidised. If rubber caps are used to close the containers they should be previously immersed in a 0.1 per cent solution of sodium metabisulphite for forty eight hours. Injection of adrenaline should be stored protected from light.

Other Adrenaline Preparations Numerous attempts have been made to devise preparations of adrenaline which can exert their effect over a prolonged period. A suspension of adrenaline in oil prepared by triturating adrenaline with sterile arachis oil has been used usually about 0.25 per cent of adrenaline is incorporated. Other preparations used for the same purpose contain combinations of adrenaline with mucic acid and with ascorbic acid.

Injection of adrenaline mucate may be prepared from the following formula using the method of the British Pharmacopœia for injection of adrenaline.

Adrenaline	0.1 g
Mucic Acid	0.114 g
Sodium Metabisulphate	0.1 g
Sodium Chloride	0.8 g
Chlorocresol	0.1 g
Water for Injection	to 100 ml

A preparation containing adrenaline ascorbate was described by Kennedy¹⁰ who gave the following formula

Adrenaline	0.01 g
Ascorbic Acid	0.015 g
White Beeswax	0.02 g
Wool Fat	0.04 g
Arachis Oil	1 ml

Heat together the white beeswax, the wool fat and a portion of the arachis oil at 150 for one hour and cool. Mix the adrenaline and the ascorbic acid and powder very finely in a sterile mortar. Triturate the powders with the remainder of the arachis oil previously heated at 150 for one hour and cooled and add the mixture of waxes.

The final injection is semi solid at ordinary temperatures and should be warmed to about 40 before use; the syringe should also be warmed to the same temperature.

Preparations for the eye include a compound lotion (Collivrium Adrenalinæ Compositum B.P.C.), and an eye ointment a suitable formula for which is

Adrenaline	0.025 g
Chlorbutol	2.0 g
Loric Acid	0.075 g
Distilled Water	2.0 ml
Wool Fat	5.0 g
Liquid Paraffin	20.0 g
White Soft Paraffin	to 100.0 g

Dissolve the adrenaline and the loric acid in the water and the chlorbutol in the liquid paraffin. Mix the wool fat with the white soft paraffin and incorporate the two solutions.

Adrenaline is administered as a nasal spray either alone (Nebula Adrenalinæ B.P.C.) or combined with atropine and papaverine (Nebula Adrenalinæ et Atropinæ Composita B.P.C.). It is also applied to the nose as a compound ointment prepared according to the following formula

Solution of Adrenaline Hydrochloride	4 ml
Chlorbutol	4 g
Benzocaine	4 g
Wool Fat	12 g
Yellow Soft Paraffin	to 100 g

Mix the wool fat and yellow soft paraffin and incorporate a mixture of the chlorbutol and benzocaine in fine powder and then the solution of adrenaline hydrochloride.

For rectal application adrenaline is used as suppositories (Suppositorium Adrenalinæ B.P.C. and Suppositorium Adrenalinæ et Cocainæ B.P.C.).

Steroid Hormones In this section the method of presenting the steroid hormones from the adrenal cortex and the gonads and the artificial oestrogens are briefly reviewed. In general these substances are all stable, crystalline and well defined and present few problems in pharmacy. Their chief interest lies in the wide range of forms in which they are used in contrast to most of the hormones previously discussed. On account of this it is convenient to arrange this section according to the route of administration.

Oral Administration In general the natural oestrogenic hormones lose much activity when given by mouth, and except for oestrone are seldom given by this route. The artificial hormones on the other hand, lose little activity and have proved effective by mouth. They are usually administered as tablets and the following are described in the British Pharmacopœia.

Tabellæ Ethisteroni
 Tabellæ Dienœstrolis
 Tabellæ Hexœstrolis
 Tabellæ Methyltestosteroni
 Tabellæ Stilbœstrolis

Tablets of oestrone (Tabellæ (Estroni) are also described in the British Pharmacopœia.

Sublingual Administration Those substances which are readily absorbed by the rich network of capillaries under the tongue can be introduced directly into the systemic circulation without risk of destruction by the enzymes of the alimentary tract or the liver. Among the substances thus administered are ethisterone and methyltestosterone, which are supplied in special tablets for sublingual administration. They should be allowed to dissolve slowly under the tongue as little as possible of the saliva being swallowed. Deoxycortone acetate has also been administered sublingually as a 0.2 per cent solution in propylene glycol.

Percutaneous Administration The sex hormones are readily absorbed through the intact skin when applied as ointments or solutions. Several ointment bases have been tried and the most suitable appear to be hydrous ointment and ointment of wool alcohols although an oil in water base such as emulsifying ointment is also used. Stilbœstrol and dienœstrol are also absorbed through the skin particularly through the breasts and are frequently administered in this way.

A selection of suitable formulæ are given below.

Dienœstrol Cream

Dienœstrol	5.0 g
Propionic Acid	2.0 g
Oleic Acid	20.0 g
Wool Fat	7.5 g
Arachis Oil	to 100.0 ml

Mix the acids and 75 ml. of the arachis oil, add the dienœstrol and gently heat until dissolved. Add the wool fat, allow to cool and adjust to volume with the arachis oil.

Estrad. of Ointment

Estradiol Monobenzoate	0.1 g
Arachis Oil	10.0 g
Hydrous Wool Fat	to 100.0 g

Dissolve the estradiol monobenzoate in the arachis oil with the aid of gentle heat and incorporate the solution in the hydrous wool fat.

Estrone ointment may be prepared in a similar manner.

Stilboestrol Cream

(a) Stilboestrol	5.0 g
Oleic Acid	10.0 ml
Wool Fat	7.5 g
Arachis Oil	to 100.0 ml

Mix the oleic acid and 50 ml of the arachis oil, add the stilboestrol and gently heat until dissolved. Add the wool fat, allow to cool and adjust to volume with arachis oil.

(b) Stilboestrol	0.2 g
Emulsifying Wax	10.0 g
Arachis Oil	30.0 g
Distilled Water	to 100.0 g

Melt the emulsifying wax in the arachis oil, add the stilboestrol and continue heating until solution is effected. Allow to cool and add the distilled water in small portions shaking between each addition.

Oily solutions are also used for percutaneous administration. Deoxycortone acetate is sometimes given by rubbing a 0.02 per cent solution in benzyl alcohol into the skin of the thigh, arm or abdomen.

Vaginal and Rectal Administration. Estradiol, estrone and the artificial oestrogens are used as pessaries, the basis being either oil of theobroma or glycerin suppository masses of the British Pharmacopoeia. The esters of the hormones are soluble in oil of theobroma and may be made in the usual way. When glycerin suppository mass is used the hormones should be dissolved in propylene glycol before incorporation since they are not very soluble in water.

The only hormone which appears to have been administered rectally is testosterone. Suppositories of testosterone are used to introduce the hormone into the blood stream and not for a local effect. They usually have an oil of theobroma base.

Nasal Administration. Estrone has been used as a solution in arachis oil containing 100 micrograms in a millilitre for local nasal application¹¹. Stilboestrol has been used similarly as a 0.1 per cent solution in arachis oil.

Parenteral Administration. The naturally occurring sex hormones are chiefly administered by intramuscular injection usually as the acetate, benzoate or propionate. For this purpose the esters are dissolved in a suitable oil such as arachis or olive oil or in ethyl oleate and the solution is sterilised by heating at 150° for one hour or if the volume of solution in a container exceeds 30 millilitres for a longer period of time sufficient to ensure that the whole of the contents are heated at 150° for one hour. The following injections are described in the British Pharmacopoeia.

Injectio Œstradiolis Dipropionatis
 Injectio Œstradiolis Monobenzoatis
 Injectio Progesteroni
 Injectio Testosteroni Propionatis

Deoxycortone acetate is also given intramuscularly in an oily solution prepared in a similar way (Injectio Deoxycortoni Acetatis B P). A water soluble glycosidic derivative of deoxycortone for intravenous injection is also available.

Artificial Œstrogens although usually given by mouth are sometimes administered by intramuscular injection. Solutions in oil for this purpose are prepared in a manner similar to that described for the naturally occurring hormones.

Aqueous solutions of natural and artificial hormones when required for injection are sterilised by heating in an autoclave. Where the solubility of the substance in water is very low a mixture of propylene glycol and water is often satisfactory as a solvent.

Deoxycortone acetate is sometimes given by intravenous injection as a 0.1 per cent solution in propylene glycol; the solution may be sterilised by heating at 150° for one hour in the final closed container.

Certain sex hormones are administered by injection as a suspension of a microcrystalline powder in water. When so injected the hormones are only slowly absorbed and therefore exert their effect over a prolonged period. Cortisone is similarly used, mainly as the acetate which is prepared in the form of microcrystals 5 to 10 microns in diameter and suspended in normal saline solution in a concentration of 25 milligrams per millilitre¹.

Implantation. The most satisfactory method of obtaining a continuous and prolonged effect is by implanting a pellet of the hormone subcutaneously. Pellets are supplied sterile in sealed glass tubes and the hormones usually administered in this way are Œstradiol, Œstrone, progesterone, testosterone and deoxycortone acetate. Methods of implantation are described on page 161. Flat implantation pellets are sometimes preferred to fused pellets.

Hormone Allergy Test Sets for diagnosis and for the desensitisation of patients with hormone allergy (see page 172) are available. For diagnosis the sets contain solutions in olive oil of cholesterol, deoxycortone acetate, Œstradiol monobenzoate, Œstrone, progesterone and testosterone dipropionate in 0.5 millilitre ampoules containing 0.005 or 0.05 milligram together with ampoules of olive oil for control. The sets for desensitisation consist of similar solutions containing 0.1 or 1.0 milligram per millilitre and are supplied in rubber capped bottles.

LEGAL REQUIREMENTS

In Great Britain a number of hormones and their preparations are subject to certain forms of legal control. Insulin and pituitary (posterior

lobe) extracts are controlled by the Therapeutic Substances Act 1925 and insulin and the active principles of the pituitary, suprarenal and thyroid glands together with the salts of the active principles of the suprarenal and thyroid glands are included in the Poisons List 1930 (S I 1930 No 1213) and various Schedules to the Poisons Rules 1949 (S I 1949 No 539)

Therapeutic Substances Act 1925 This act controls the manufacture, sale and importation of those therapeutic substances the purity or potency of which cannot be adequately tested by chemical means. The requirements of the Act are supplemented by regulations issued under the authority of the Act for hormones: the most important regulations are the Therapeutic Substances Regulations 1931 (S R and O 1931 No 533) which define the general and special requirements for insulin and pituitary (posterior lobe) extracts. These requirements specify *inter alia* the conditions under which the preparations must be manufactured, the types of container, the method of labelling and tests for strength, quality and sterility.

The containers for liquid preparations must be sealed glass containers made of non-alkaline resistance glass, and if multiple dose containers are used, the preparation must contain a bacteriostatic to prevent the growth of any organism introduced during the removal of part of the contents.

The label on the container must state the proper name of the substance as defined by the regulations, the licence and batch number and the strength in units per millilitre. The containers of insulin tablets must state the number of units in each tablet. The label on the container or the label or wrapper on the package must state the name and address of the manufacturer, the date on which the manufacture of the batch was completed, the date up to which the preparation may be expected under suitable conditions to retain its potency, the storage conditions necessary to retain this potency, and the name and proportion of any added bacteriostatic. Preparations may not be sold after the date of expiry stated on the label, except to a medical practitioner who has been acquainted with this fact and is satisfied that the sale is required by the urgency of the case.

Other special requirements for insulin forbid its issue in a mixture with any other therapeutic agent without the consent of the licensing authority, and stipulate that if issued for injection as a suspension, it shall be tested before suspension.

Pharmacy and Poisons Act 1933 The following substances are included in Part I of the Poisons List:

Insulin

Pituitary gland, the active principles of

Suprarenal gland the active principles of their salts

Thyroid gland the active principles of their salts

These substances do not appear in the First or Fourth Schedule to the Poison Rules 1949 but are included in the Sixth and Seventh Schedules

The Sixth Schedule specifies a permissible form of disclosure for the proportion of the poison, and the relevant entries are as follows

Insulin The number of units of activity as defined in the British Pharmacopœia contained in a specified quantity of the preparation

Pituitary gland the active principles of Either

(a) The number of units of activity as defined in the British Pharmacopœia contained in a specified quantity of the preparation or

(b) the proportion of pituitary gland or of anterior or of posterior lobe of the gland as the case may be contained in the preparation or

(c) the amount of pituitary gland or of anterior or of posterior lobe of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Suprarenal gland the active principles of their salts Either

(a) the proportion of suprarenal gland or of the cortex or of the medulla of the gland as the case may be contained in the preparation or

(b) the amount of suprarenal gland or of the cortex or of the medulla of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Thyroid gland the active principles of their salts Either

(a) the proportion of thyroid gland contained in the preparation or

(b) the amount of thyroid gland from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland

The Seventh Schedule gives the special wording to be used on the labels of certain preparations in place of the word *Poison*. Medicines made up ready for the internal treatment of human ailments must be labelled with the words *Caution It is dangerous to take this preparation except under medical supervision* if they contain one of the following

Insulin

Pituitary gland the active principles of

Thyroid gland the active principles of their salts

If they contain the active principles of suprarenal gland or their salts the prescribed wording is *Caution It is dangerous to exceed the stated dose*. When the medicines are not made up ready for the internal treatment of human ailments the cautionary phrases given above do not apply and the word *Poison* must be used instead

When either of the cautionary phrases or the word *Poison* is used it must not be modified in meaning by adding any other words or marks and it must be either on a separate label or surrounded by a line within which there must be no words other than those required by law

REFERENCES

- 1 LEYTON O and POULTON E P *Lancet* 1931 : 996
- 2 BENNETT T I *Lancet* 1931 : 1053

- 3 SANDOR F *Lancet* 1931 i 1421
- 4 HARTLEY P *Lancet* 1931 i 582
- 5 LYONS W R *Endocrinology* 1941 28 161
- 6 REISS M and HALKERSTON I D K *J Pharm Pharmacol* 1950 2 236
- 7 COURT D and TAYLOR S A *Lancet* 1943 i 265
- 8 COURT D and TAYLOR S A *Pharm J* 1939 2 219
- 9 WEST G *Quart J Pharm* 1945 18 267 299 1946 19 256 392
- 10 KENNEDY A *Lancet* 1941 ii 279
- 11 COLLIP J B MORTIMER, H and WRIGHT R P *Canad med Ass J* 1937 37 445
- 12 HENCH P S KENDALL E G SLOCUM C H and POLLEY H F *Proc Mayo Clin* 1949 24 181

Suprarenal gland, the active principles of their salts

Thyroid gland the active principles of their salts

These substances do not appear in the First or Fourth Schedule to the Poison Rules 1949 but are included in the Sixth and Seventh Schedules

The Sixth Schedule specifies a permissible form of disclosure for the proportion of the poison and the relevant entries are as follows

Insulin The number of units of activity as defined in the British Pharmacopoeia contained in a specified quantity of the preparation

Pituitary gland the active principles of Either

- (a) The number of units of activity as defined in the British Pharmacopoeia contained in a specified quantity of the preparation or
- (b) the proportion of pituitary gland or of anterior or of posterior lobe of the gland as the case may be contained in the preparation or
- (c) the amount of pituitary gland or of anterior or of posterior lobe of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Suprarenal gland the active principles of their salts Either

- (a) the proportion of suprarenal gland or of the cortex or of the medulla of the gland as the case may be contained in the preparation or
- (b) the amount of suprarenal gland or of the cortex or of the medulla of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Thyroid gland the active principles of their salts Either

- (a) the proportion of thyroid gland contained in the preparation or
- (b) the amount of thyroid gland from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland

The Seventh Schedule gives the special wording to be used on the labels of certain preparations in place of the word *Poison*. Medicines made up ready for the internal treatment of human ailments must be labelled with the words *Caution It is dangerous to take this preparation except under medical supervision* if they contain one of the following

Insulin

Pituitary gland the active principles of

Thyroid gland the active principles of their salts

If they contain the active principles of suprarenal gland or their salts the prescribed wording is *Caution It is dangerous to exceed the stated dose*. When the medicines are not made up ready for the internal treatment of human ailments the cautionary phrases given above do not apply and the word *Poison* must be used instead.

When either of the cautionary phrases or the word *Poison* is used it must not be modified in meaning by adding any other words or marks and it must be either on a separate label or surrounded by a line within which there must be no words other than those required by law.

REFERENCES

- 1 LEYTON O and POULTON E P *Lancet* 1931 : 996
- 2 BENNETT T I *Lancet* 1931 : 1053

Adrephne Ampoules	1 ml ampoules containing adrenalin 1 in 2500 ephedrine hydrochloride 3 per cent and Chloretone 0.5 per cent	Parke Davis
Epphetonogen	1 ml ampoules containing adrenalin $\frac{1}{100}$ gr and ephedrine hydrochloride $\frac{1}{2}$ gr	Richter
Epphetonogen Forte	1 ml ampoules containing adrenalin $\frac{1}{25}$ gr ephedrine hydrochloride 4 gr and atropine $\frac{1}{100}$ gr	Richter
Hyperduric Adrenalin	$\frac{1}{2}$ and 1 ml ampoules and 5 ml vials containing adrenalin 1 in 1000 as the mucate	Allen and Hanburys
Pitalin	1 ml ampoules containing injection of pituitary (posterior lobe) 0.5 ml and injection of adrenalin 0.36 ml	Paines and Byrne
Pitrenalin	10 ml ampoules containing 0.5 ml of pituitrin and 0.36 ml of adrenalin chloride solution (1 in 1000)	Parke Davis
Suprarenalin	1 ml ampoules containing adrenalin 1 in 1000	Armour
Vaso-constrictine	1 ml ampoules and 30 ml vials containing adrenalin 1 in 1000	Duncan Flockhart
Ointments		
Adrenalin and Chloretone Eye Ointment	Adrenalin 1 in 4000 with Chloretone 5 per cent	Parke Davis
Adrenalin and Chloretone Ointment	Adrenalin 1 in 1000 with Chloretone 5 per cent	Parke Davis
Adrenalin Ointment	Adrenalin 1 in 1000	Parke Davis
Anusar	Contains adrenalin phenol and zinc oxide	Allen and Hanburys
Vaso-constrictine Ointment	Adrenalin 1 in 1000	Duncan Flockhart
Solutions		
(Solution of Adrenalin B.P. contains adrenalin 1 in 1000 in 10 ml vials and $\frac{1}{2}$, 1, 2 and 4 fl oz bottles)		It is supplied in 10 ml
Adrenalin Borate Solution	1 fl oz bottles	British Drug Houses
Adrephne	10 ml vial and 1 fl oz bottles containing adrenalin 1 in 1000 ephedrine sulphate 2 per cent and Chloretone 0.5 per cent	Parke Davis
Suprarenalin	1 fl oz and 1 litre bottles containing adrenalin 1 in 1000	Armour
Vaso-constrictine	1 fl oz bottles containing adrenalin 1 in 1000	Duncan Flockhart
Suppositories		
(Adrenalin Suppositories B.P.C. contain adrenalin $\frac{1}{100}$ gr)		
Adrenalin and Chloretone Suppositories	Adrenalin 1 in 1000 with Chloretone 3 gr	Parke Davis
Adrenalin Suppositories	Adrenalin hydrochloride $\frac{1}{25}$ gr equivalent to adrenalin 1 in 1000	Parke Davis
Anusar	Contains adrenalin phenol and zinc oxide	Allen and Hanburys

CHAPTER VIII

COMMERCIAL PREPARATIONS

MOST of the hormones and related substances which have been described in earlier pages are obtainable in a variety of pharmaceutical forms sometimes under official or accepted names and sometimes under trade names. The following list gives the names of a number of these preparations and the suppliers together with a brief description where necessary of the composition strength and type and size of container the full names and addresses of the suppliers will be found at the end of the chapter. The list is not comprehensive and many of the preparations of the British Pharmacopœia and British Pharmaceutical Codex may be obtained by pharmacists from their usual drug suppliers under the official titles.

The preparations are grouped according to the substance they consist of or contain the substances concerned being arranged alphabetically under the gland or part of gland to which they correspond the glands themselves are also arranged alphabetically.

ADRENALS

Adrenaline

Inhalants

Adrenaline Chloride Solution 1 in 100	5 ml vials	Larke Davis
Adrenalin Inhalant	1 fl oz bottles containing adrenaline 1 in 1000 with Chlorotone	Parke Davis
Adrenaline Inhalant	5 and 10 ml bottles containing adrenaline 1 in 100	Burroughs Wellcome
Adrenaline Solution 1 in 100 (for inhalation)	5 ml and 1 fl oz vials	Evans Oxo
Adrenaline Tartrate Spray Solution	15 ml vials	Paines and Byrne
Adrephine Inhalant	10- and 25 ml bottles containing adrenaline 1 in 1000	Burroughs Wellcome
	10 ml vials containing adrenaline 1 in 10 000 ephedrine hydrochloride 1 per cent benzocaine 1 per cent and Chlorotone 0.5 per cent in glycerin	Parke Davis

Injections

(Injection of Adrenaline B.P. contains adrenaline 1 in 1000 it is supplied in 0.5, 1 and 2 ml ampoules and in 5, 10, 15, 20 and 30 ml vials)		
Adrenalin Chloride Solution	0.5 and 1 ml ampoules 10 ml vial and 1 fl oz bottles containing adrenaline 1 in 1000 1 ml ampoules containing adrenaline 1 in 10 000	Parke Davis
Adrenalin in Oil	1 ml ampoules containing 2 mg	Parke Davis
Adrenaline Suspension with Ascorbic Acid	10 ml vials containing adrenaline 1 in 100	British Drug Houses
Adrenutol	Injectable suspension containing 1 or 2 mg per ml	Evans

Tablets

(Tablets of Methyltestosterone B.P. contain 5 mg unless otherwise ordered)

Methyltestosterone	5 or 10 mg	Boots
	■ 10 25 or 50 mg	{ British Drug Houses
	■ mg	{ Oxo
Erugon S Tablets	5 mg	Burroughs Wellcome
Gloss Sterandryl	5 10 25 or 50 mg	Bayer
Neo-Hombreol (M)	5 10 25 or 50 mg	Roussel
Oxavron	■ 10 25 or 50 mg	Organon
Perandren Linguets	5 10 25 or 50 mg	Peitlich Schering
Virormone Oral	5 10 25 or 50 mg	Ciba
		Paines and Byrne

*Testosterone**Implantation Tablets*

Testosterone	25 50 100 150 200 300 or 350 mg	Organon
--------------	---------------------------------	---------

Injections

Virormone	Ampoules containing 5 10 25 50 100 or 250 mg	Paines and Byrne
Virormone N	2 ml ampoules containing 50 mg in aqueous suspension	Paines and Byrne

Oral Tablets

Perandren	2 mg per g	Ciba
Virormone Soluble	2 mg per g	Paines and Byrne

Suppositories

Virormone	15 mg	Paines and Byrne
-----------	-------	------------------

*Testosterone Propionate**Implantation Tablets*

Perandren Implants	100 mg	Ciba
Sterandryl Implant	100 mg	Roussel
Testosterone	100 mg	{ British Drug Houses
Propionate	25 50 100 150 200 300 or 350 mg	{ Organon

Injections

(Injection of Testosterone Propionate B.P. contains 10 mg per ml unless otherwise ordered)

Testosterone	1 ml ampoules containing 5 10 or 50 mg	{ Boots
Propionate	1 ml ampoules containing 1 5 10 25 or 50 mg 2 ml ampoules containing 100 mg	{ Burroughs Wellcome
	1 ml ampoules containing 5 10 25 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 500 mg	{ Oxo
	1 ml ampoules containing 5 10 25 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 500 mg	British Drug Houses
Erugon S	Ampoules containing 5 10 or 25 mg	Bayer
Neo-Hombreol	1 ml ampoules containing 5 10 5 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 50 mg per ml	{ Organon
Pantestun	Ampoules containing 5 10 or 25 mg	Richt
Perandren	1 ml ampoules containing 5 10 25 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 50 mg per ml	Ciba

Tablets

Adrenalin and Cocaine Hypodermic Tablets	Adrenaline 0.05 mg cocaine hydrochloride 0.01 g	Parke Davis
Adrenalin Hypodermic Tablets	0.3 or 1 mg	Parke Davis
Adrenaline Tablets	1/100 or 1/50 gr	Faines and Byrne
Suprarenalin	1/100 or 1/50 gr	Armour
Vaso constrictive Hypotabs	Adrenaline 1/100 gr	Duncan Flockhart

Adrenal Cortex**Cortical Extracts**

Cortigen	1 ml ampoules and 10 and 30 ml vials	Richter
Eschatin	10 ml vials containing 200 µg of cortisone per ml	Parke Davis
Eucortone	10 ml vials	Allen and Hanburys
Supracort	Each ml is equivalent to 75 g of fresh gland 1 ml ampoules and 10 ml vials	Faines and Byrne
Suprarenal Cortex Extract	10 ml vials	British Drug Houses

Deoxycortone Acetate*Implantation Tablets*

D O C A	25 50 100 150 200 or 300 mg	Organon
Deoxycortone Acetate	100 mg	British Drug Houses
Percorten Implants	100 mg	Ciba
Syn cortyl Implants	100 mg	Roussel

Injectons

(Injection of Deoxycortone Acetate H.P. contains 5 mg per ml unless otherwise ordered)

Deoxycortone Acetate	1 ml ampoules containing 5 or 10 mg 10 ml vials containing 10 mg per ml	British Drug Houses
Cortison	1 ml ampoules containing 2.5 or 10 mg	British Schering
D O C A	1 ml ampoules containing 2.5 or 10 mg 5 ml vials containing 5 mg per ml	Organon
Percorten	1 ml ampoules containing 2.5 or 10 mg 10 ml vial containing 10 mg per ml	Ciba
Percorten Crystals	2 ml ampoules containing 50 mg in aqueous suspension with 2 mg of Nupercaine	Ciba
Percorten Water Soluble	1 ml ampoules containing deoxycortone glucoside in aqueous solution	Ciba
Syn cortyl	1 ml ampoules containing 5 or 10 mg	Roussel

Sublingual Tablets

D O C A	1 mg	Organon
Percorten Langlets	1 mg	Ciba

GONADS**Androgens****Methyltestosterone***Injection*

Micryston Methyltestosterone	8 ml vials containing 5 mg per ml suspended in normal saline	Coates and Cooper
------------------------------	--	-------------------

Hexoestrol*Injectables*

Hexoestrol	1 ml ampoules containing 1 or 5 mg	{ Boots British Drug Houses Paines and Byrne
------------	------------------------------------	--

Tablets

(Tablets of Hexoestrol B.P. contain 1 mg unless otherwise ordered)

Hexoestrol	1 or 5 mg	{ Boots Barringtons Wellcome British Drug Houses
	0.5 1 or 5 mg	{ Oxo Organon
	0.1 0.5 1 or 5 mg	{ Paines and Byrne Ortho Pharmaceuticals
Hexital	3 mg with 20 mg of phenobarbital	Carrick
Thelgestrol	3 mg with 20 mg of phenobarbital	

Hexoestrol Dipropionate*Injectables*

Hexoestrol Dipropionate	1.5-ml ampoules containing 15 mg	Barringtons Wellcome
-------------------------	----------------------------------	----------------------

Öestradiol*Implants or Tablets*

Gynosteryl Implants	20 mg	Roussel
Öestradiol Implantation Pellets	10 15 20 25 50 or 100 mg	Organon
Östroform Pellets	20 mg	British Drug Houses
Ovocyclin Implants	20 mg	Ciba

Ointment

Ovocyclin	0.1 mg per g	Ciba
Progyon	0.1 mg per g	British Serravallo

Pessaries

Progyon Vaginal Suppositories	0.36 mg	Perrin Schering
-------------------------------	---------	-----------------

Sublingual Tablets

Ovocyclin Linguets	0.04 0.1 or 1 mg	Ciba
--------------------	------------------	------

Tablets

Glandubol n	0.1 0.5 or 1 mg	Richter
-------------	-----------------	---------

Öestradiol Dipropionate*Injectables*

(Injection of Öestradiol Dipropionate B.P. contains 1 mg per ml unless otherwise ordered.)

Dimenformon Dipropionate	5-ml vials containing 25 mg in oil	Organon
Glandubol n	1 ml ampoules containing 0.1 1 or 5 mg	Richter
Ovocyclin P	1 ml ampoules containing 1 or 5 mg in oil	Ciba
Progyon DP	1 ml ampoules containing 20.000 mg in oil	British Serravallo

HORMONES

Levandren Crystals	2 ml ampoules containing 50 mg, in aqueous suspension with 2 mg of Nupercaine	Ciba
Levandrel	1 ml ampoules containing 10, 25 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 500 mg	Roussel
Testoviron	1 ml ampoules containing 5, 10 or 25 mg	British Schering
Valotest	1 ml ampoules containing 5, 10 or 25 mg	Wallace
<i>Ointments</i>		
Neo-Himbreol	2 or 25 mg per g	Organon
Levandren	2 mg per g	Ciba
Testosterone Ointment	2.5 per cent	Boots
Testoviron	2 mg per g	British Schering
<i>Suppositories</i>		
Neo-Himbreol	15 mg	Organon

Oestrogens

*Dienoestrol**Ointment*

Dienoestrol Ointment	2.5 per cent	British Drug Houses
----------------------	--------------	---------------------

Tablets

(Tablets of Dienoestrol B.P. contain 0.1 mg unless otherwise ordered)

Dienoestrol	0.1, 0.3, 1 or 5 mg	Allen and Hanburys Boots British Drug Houses Oxo Faines and Byrne Organon Armour
Armo-Naestrol	0.03, 0.1, 0.3, 1 or 5 mg 0.1 mg with 10 mg of phenobarbitone	Armour
Armo-Naestrol Forte	0.3 mg with 16 mg of phenobarbitone	Armour

*Ethinylœstradiol**Elvis*

Estigyn	0.02 mg per 60 m	British Drug Houses
---------	------------------	---------------------

Tablets

(Tablets of Ethinylœstradiol B.P. contain 0.02 mg unless otherwise ordered)

Amenorone	0.01 mg with 10 mg of ethisterone	Roussel
Barboestrol	0.01 mg with 16 mg of phenobarbitone and of papaverine	Roussel
Estigyn	0.01, 0.05 or 1 mg	British Drug Houses
Ethidol	0.01 or 0.05 mg	British Schering
Ethin (Estyl)	0.01, 0.05 or 1 mg	Roussel
Ethinylœstradiol	0.01 or 0.05 mg	Armour
	0.01, 0.05, 0.1 or 1 mg	Oxo
	0.01, 0.05 or 1 mg	Faines and Byrne
Eucyclin	1 mg	Ciba
Eucyclin Linguets	0.01 or 0.05 mg	Ciba
Lynoral	0.01, 0.05 or 1 mg	Organon
Orasecron	0.05 mg with 10 mg of ethisterone	British Schering

Ointments

Menformon	0.5 mg per g	Organon
Estroform	1000 i.u. per g	British Drug Houses
Estrosal	10 000 i.u. per oz	Paines and Byrne
Estrosalve Concentrated	2500 i.u. per g	Paines and Byrne
Unden	5000 i.u. per g	Bayer

Pessaries

Kolpon Vaginal Bougies	0.1 mg	Organon
Estroform	1000 i.u. (adult or child size)	British Drug Houses
Estrone	1000 or 5000 i.u.	Paines and Byrne

Solutions

Menformon	10-ml bottles containing 1 mg per ml for oral use	Organon
Oleum Ketodestrin	Only solution containing 5000 i.u. per g	Paines and Byrne
Sol Ketodestrin	1-oz bottles of aqueous solution containing 1000 i.u. per 60 ml	Paines and Byrne
Solestrin	15- and 30-ml bottles of alcoholic solution containing about 1300 i.u. per drop	Paines and Byrne

Suspensions

Menformon	0.1 or 1 mg	Organon
-----------	-------------	---------

Tablets

(Tablets of Estrone B.P.)	contain 1 mg unless otherwise ordered)	
Menformon	0.05 0.1 0.3 1 or 5 mg	Organon
Estrin	1000 i.u.	Oxo
Estroform	1000 5000 or 10 000 i.u.	British Drug Houses
Estrone	500 1000 3000 5000 or 10 000 i.u.	Paines and Byrne
Progynon	1000 3000 or 10 000 i.u.	British Schering
Strogene	1 mg of conjugated oestrogens calculated as sodium oestrone sulphate	Wyeth
Unden Pellets	100 500 or 1000 i.u.	Bayer

*Stilboestrol**Injections*

Pabestol	1 ml ampoules containing 1 or 5 mg	Paines and Byrne
Stilbestol	1 ml ampoules containing 1 or 5 mg	{ Allen and Hanburys Boots British Drug Houses Burroughs Wellcome Crookes
	1 ml ampoules containing 5 ml	
	0.5-ml ampoules containing 0.5 mg 1 ml ampoules containing 1 or 5 mg	

Ointments

Pabestrosal	Ointment containing 2 per cent with benzocaine 2.5 per cent	Paines and Byrne
Stilboestrol Ointment	0.5 or 1 per cent	Boots
Stilboestrol Salve	0.1 mg per g	Crookes

Pessaries

Stilboestrol and Lactic Acid	Stilboestrol 1 mg with lactic acid 1 per cent	Crookes
------------------------------	---	---------

Sprays

Stilboestrol Spray	0.5 mg per ml in oil	Crookes
--------------------	----------------------	---------

Œstradiol Monobenzoate*Injections*

(Injection of Œstradiol Monobenzoate H.P. contains 1 mg per ml unless otherwise ordered)

Benzo Gynöstryl	1 ml ampoules containing 1 or 5 mg	Roussel
Benztrone	1 ml ampoules containing 1·2 or 5 mg	Paines and Byrne
Disecron	1 ml ampoules and 10 ml vials containing 2·5 mg with 12·5 mg of progesterone per ml	British Schering
Dimenformon	1 ml ampoules and 5 ml vials containing 10·000 i.u. per ml 1 and 2 ml ampoules and 5 and 10 ml vials containing 50·000 i.u. per ml	Organon
Gynformon	1 ml ampoules containing 10·000 i.u.	Wallace
Gynformone Frée	1 ml ampoules containing 50·000 i.u.	Wallace
Œstrin	1 ml ampoules containing 1000, 10·000, 20·000 or 50·000 i.u.	Ovo
Œstroform	1 ml ampoules containing 1000, 10·000, 20·000 or 50·000 i.u.	British Drug Houses
Ovocyclin B	1 ml ampoules containing 1 or 5 mg in oil	Ciba
Ovocyclin B Crystals	20 ml ampoules containing 10 mg in aqueous suspension with 2 mg of Nupercaine	Ciba
Progynon B Oleosum	1 ml ampoules and 5 and 10 ml vials containing 10·000 or 50·000 i.u. per ml	British Schering
Uden	1 ml ampoules and 10-ml bottles containing 10·000 i.u. per ml 1 ml ampoules containing 50·000 i.u.	Bayer

Ointment

Dimenformon	2 mg per g	Organon
-------------	------------	---------

Œstriol*Capsules*

Theolol	0·125 mg	Parke Davis
---------	----------	-------------

Œstrone*Injections*

Ketodestrin	1 ml ampoule containing 0·05, 0·1, 0·5 and 1 mg	Paines and Byrne
Menformon	1 ml ampoules containing 0·1 mg (aqueous) 1 ml ampoules and 5 ml vials containing 1 mg per ml in an organic solvent	Organon
Micryston Œstrone	8 ml vial containing 1 mg per ml suspended in normal saline	Coates and Cooper
Theelin (Aqueous Suspension)	1 ml ampoules containing 2 mg	Parke Davis
Theelin in Oil	1 ml ampoules containing 0·1, 0·2, 0·5 or 1 mg	Parke Davis
Uden	1 ml ampoules containing 1000 i.u. in aqueous solution	Bayer

Injections

Corpus Luteum Extract	5-ml ampoules and 10- and 20-ml vials (1 ml is equivalent to about 3 gr of dried gland)	Armour
	0.5- and 1-ml ampoules and 10- and 20-ml bottles (1 ml is equivalent to 2 gr of dried gland)	Oxo
	1-ml ampoules containing the equivalent of 1 g of fresh gland	Paines and Byrne

Tablets

Corpus Luteum	$\frac{1}{2}$, 1, 2 or 5 gr	Armour
	1, 2 or 5 gr	Paines and Byrne
Corpus Luteum Substance Granules	0.1296 g of dried gland	Armour
Luteomycin	Tablets containing $\frac{1}{2}$ or $\frac{1}{4}$ gr of extract	Paines and Byrne

Ethisterone

(Tablets of Ethisterone B.P. contain 5 mg unless otherwise ordered)

Ethisterone	5 or 10 mg	Boots
	5 mg	Burroughs Wellcome
	5, 10 or 25 mg	British Drug Houses
Gestone Oral	5, 10 or 25 mg	Paines and Byrne
Lutocyclin Linguets	5, 10 or 25 mg	Ciba
Lutogyl Oral	5, 10 or 25 mg	Roussel
Oraluton	5, 10 or 25 mg	British Schering
Progesterol	5, 10 or 25 mg	Organon

*Progesterone**Implantation Tablets*

Lutocyclin Implants	100 mg	Ciba
Lutogyl Implants	100 mg	Roussel

Injections

(Injection of Progesterone B.P. contains 5 mg per ml unless otherwise ordered)

Progesterone	1-ml ampoules containing 2.5 or 10 mg	Boots
	1-ml ampoules containing 1, 2.5, 10, 25 or 50 mg	Burroughs Wellcome
	1-ml ampoules containing 5 or 10 mg; 10-ml vials containing 5 mg per ml	Oxo
	1-ml ampoules containing 5 or 10 mg; 10-ml vials containing 5 mg per ml	Parke Davis
Disecron	1-ml ampoules and 10-ml vials containing 12.5 mg with 2.5 mg of oestradiol monobenzoate per ml	British Schering
Gestone	1-ml ampoules containing 1, 2.5, 10 or 25 mg	Paines and Byrne
Glanducorpin	2.5- or 10-mg ampoules	Richter
Gynoluton	1-ml ampoules containing 11 mg	Wallace
Gynolutone Forte	1-ml ampoules containing 5 mg	Wallace
Luteomycin	1-ml ampoules containing 1 mg	Paines and Byrne
Lutocyclin	1-ml ampoules containing 2.5, 10 or 25 mg; 10-ml vials containing 25 mg per ml	Ciba
Lutocyclin Crystules	1-ml ampoules containing 50 mg with 2 mg of Nupercaine	Ciba
Lutogyl	1-ml ampoules containing 2.5 or 10 mg	Roussel
Lutren	1-ml ampoules containing 2 or 5 mg	Bayer

Tablets

(Tablets of Stilboestrol H P contain 0.1 mg unless otherwise ordered)

Stilboestrol Tablets	0.5, 1 or 5 mg	{ Allen and Hanburys British Drug Houses Burroughs Wellcome Oxo
	0.1, 0.5, 1 or 5 mg	{ Boots Organon
Enseals Diethylstilboestrol	0.1 or 5 mg	Crookes
Ovendosyn	0.5 or 1 mg	Lilly
Ovendosyn Forte	Tablets containing 0.5 mg with calcium phosphate 290 mg	Menley and James
Pabestrol	Tablets containing 5 mg with calcium phosphate 325 mg	Menley and James
Pabestrol EC	0.05, 0.1, 0.5, 1 or 5 mg	Paines and Byrne
	Enteric coated tablets containing 0.5, 1 or 5 mg	Paines and Byrne
Sedestrol	Tablets containing 0.1 mg with 16 mg of phenobarbitone	Geigy
Stilboestrol Compound	Tablets containing 1 mg with 16 mg of phenobarbitone	Boots

Stilboestrol Dipropionate*Injectons*

Pabestrol D	1 ml ampoules containing 1 or 5 mg	Paines and Byrne
Stilboestrol Dipropionate	1 ml ampoules containing 1 or 5 mg	{ Boots British Drug Houses Organon Crookes
	0.5-ml ampoules containing 0.5 mg, 1 ml ampoules containing 1 or 5 mg	
	Only solution ampoules containing 1 or 5 mg, 25 ml bottles containing 10 mg	Oxo
Syntestrin	Ampoules containing 1 or 5 mg	Richter

Ointment

Stilbofax	0.05 per cent	Burroughs Wellcome
Syntestrin	30 mg per oz	Richter

Suppositories

Syntestrin	1 mg	Richter
------------	------	---------

Tablets

Pabestrol D	0.1, 0.5, 1 or 5 mg	Paines and Byrne
Stilboestrol	0.1, 0.5, 1 or 5 mg	Boots
Dipropionate	0.5, 1 or 5 mg	British Drug Houses
	0.1, 1 or 5 mg	Crookes
Syntestrin	0.1, 0.25, 0.5, 1 or 5 mg	Richter

Triphenylethylene Derivatives

Gynosone	Tablets containing 0.5 g of triphenylchloroethylene	Imperial Chemical (Pharmaceuticals)
----------	---	-------------------------------------

Progestogens***Corpus Luteum***

Corpus Luteum Powder	$\frac{1}{2}$ 1 or 4 oz	Armour
----------------------	-------------------------	--------

Capsules

Corpus Luteum	2.5 or 10 gr	Armour
---------------	--------------	--------

Miscellaneous

Ambion A	1 ml ampoules containing the equivalent of 100 to 300 guinea pig units of thyrotrophic hormone and about 10 synergic rat units of gonadotrophic hormone supplied with ampoules containing 100 units of chorionic gonadotrophin	Organon
Ambion B	Similar to Ambion A but without the chorionic gonadotrophin	Organon
Antephysan	1 ml ampoules of extract	Richter
Anterior Pituitary Extract	1 ml ampoules	Parke Davis
Antoxilin	0.5- and 1 ml ampoules and 10- and 20-ml vials containing the equivalent of 2 gr of dried gland per ml	Oxo
Pitexan Snuff	Anterior lobe powder	Paines and Byrne
Pituitary Anterior Lobe	1 ml ampoules and 10- and 20-ml vials containing the equivalent of 1½ gr of dried gland	Armour
	1 ml ampoules containing the equivalent of 2 g of fresh gland	Paines and Byrne
Synapodion	10-ml vials containing a combination of the follicle stimulating hormone from the anterior lobe and chorionic gonadotrophin contains 15 synergic rat units per ml	Parke Davis

Gonadotrophins from Other Sources*Chorionic Gonadotrophin*

Antoxilin (S)	Ampoules containing 100 or 500 i.u.	Oxo
Antutrin S (Antrodin)	10-ml vials containing 100 i.u. per ml 5 ml vials containing 500 i.u. per ml	Parke Davis
Glanduantin	Ampoules containing 100 or 500 units	Richter
Gonadotrophin L.H.	Ampoules containing 100 500 1000 or 1500 i.u.	Paines and Byrne
Gonadyl Chorionic Gonad	Ampoules containing 1000 i.u.	Roussel
Physostab	Ampoules containing 100 or 500 i.u.	British Drug Houses
	Ampoules containing 500 or 1000 i.u.	Boots
Pregnyl	Ampoules containing 100 500 or 1500 i.u.	Organon
Profan	Ampoules containing 100 500 or 2000 i.u.	Bayer
Synapodion	10-ml vial containing a combination of the follicle stimulating hormone from the anterior lobe and chorionic gonadotrophin contains 15 synergic rat units per ml	Parke Davis

Serum Gonadotrophin

Antostab	Ampoules containing 500 or 1500 i.u.	Boots
Gonadyl	Ampoules containing 200 400 1000 or 3000 i.u.	Organon
Gonadotrophin F.S.H.	Ampoules containing 400 1000 1500 or 3000 i.u.	Paines and Byrne

HORMONES

Micryston	8 ml vials containing 12.5 mg	Coates and Cooper
Progesterone	per ml suspended in normal saline	
Progestin	1 ml ampoules containing 12.5	British Drug Houses
	10 or 25 mg 10 ml vials containing 25 mg per ml	
	1 ml ampoules containing 12.5	Organon
	10 or 25 mg 5 ml vials containing 2.5 or 10 mg per ml	
	10 ml vials containing 1 or 25 mg per ml	
Proluton	1 ml ampoules containing 2.5 or 10 or 25 mg	British Schering

PANCREAS

Injection of Insulin

Insulin	20 40 or 80 units per ml	{ Allen and Hanburys Boots
Unmodified Insulin	20 40 or 80 units per ml	{ British Drug Houses Burroughs Wellcome

Injection of Globin Zinc Insulin

Globin Insulin (with Zinc)	40 or 80 units per ml	{ Allen and Hanburys Boots
		{ British Drug Houses Burroughs Wellcome

Injection of Protamine Zinc Insulin

Protamine Zinc Insulin	40 or 80 units per ml	{ Allen and Hanburys Boots
		{ British Drug Houses Burroughs Wellcome

Other Insulin Preparations

Delay Insulin A B	Protamine insulin with solution of sodium phosphate to be added immediately before use 40 and 80 units per ml	British Drug Houses
Insulin Ointment	1 or 10 units per g	Organon

PARATHYROID

Dihydrotachysterol
V T 10

15 ml vials containing 125 mg per ml in oil	Bayer
Capsules containing 0.625 mg in oil	

Parathyroid Extract

Parathormone	5 ml vials containing 20 units per ml	Lilly
Paroidin	5 ml vials containing 100 units per ml	Parke Davis
Parathyroid Hormone	5 ml vials containing 20 units per ml	Paines and Byrne

PITUITARY

Anterior Lobe

Adrenocorticotrophic Hormone

Cortrophin	—	Organon
------------	---	---------

Growth Hormone

Antuitrin Growth	20 ml vials containing 8 rat growth units per ml	Parke Davis
------------------	--	-------------

Methylthiouracil	50- 100 or 200-mg tablets	{British Drug Houses Imperial Chemical (Pharmaceutical) Organon Burroughs Wellcome Genatosan
	50- or 200 mg tablets	
	100 or 200 mg tablets	
(Tablets of Methylthiouracil B.P. contain 0.1 g unless otherwise ordered)		
Propylthiouracil	25 mg tablets	Genatosan
(Tablets of Propylthiouracil B.P. contain 25 mg unless otherwise ordered)		

Thyroxine and Related Substances

D-iodotyrosine	100-mg tablets	Organon
	4 and 14 gr tablets	Paine and Byrne
Elityran Tablets	0.15 mg organic iodine and 0.09 mg thyroxine equivalent to 90 mc of thyroid	Bayer
Thyroglobulin Tablets	4 gr	Oxo
Thyroxine	Tablets containing 0.3 or 1 mg of DL thyroxine	British Schering
	Tablets containing 0.2 or 0.8 mg of DL thyroxine sodium hypoder- mic tablets 1 mg	British Drug Houses
Thyroxine sodium	1 g or 10-g bottles	Burroughs Wellcome
L-Thyroxine sodium	Tablets containing 0.05 or 1 mg of L-thyroxine sodium	Glaxo

Whole Gland

Thyroid is supplied in tablets ranging in content from $\frac{1}{16}$ gr to 5 gr by various manufacturers

Liquor Thyroides	Represents 20 per cent of fresh substance	Oppenheimer
Paromin	Enteric coated tablets containing $\frac{1}{16}$ gr of organically combined iodine and $\frac{1}{4}$ gr of thyroid	Paine and Byrne
Thyranon	Tablets containing $\frac{1}{4}$, $\frac{1}{2}$ or 2 gr	Organon
Thycolol	Capsules containing active colloid equivalent to 5 gr of thyroid	Oppenheimer

MANUFACTURERS

The full names and addresses of the manufacturers of the preparations in the above list are as follows

Allen and Hanbury Ltd Petham Green London E 2
 Armour Laboratories Ltd Lindsey St London E C 1
 Bayer Products Ltd Africa House Kingsway London W C 2
 Boots Pure Drug Co Ltd Station Street Nottingham
 British Drug Houses Ltd Gresham Street City Road London N 1
 British Schering Ltd 229-231 Kensington High Street London W 8
 Burroughs Wellcome and Co 183-193 Easton Road London N W 1
 G W Carrick and Co Newark New Jersey (Distributors Brooks and Warburton
 Ltd 740 Vauxhall Bridge Road London S W 1)
 Ciba Laboratories Ltd Horsham Sussex
 Coates and Coope Ltd Plymouth Works West Drayton Middlesex
 Coopers Laboratories Ltd Park Royal London N W 10
 Duncan Flockhart and Co Ltd 104-108 Hylford Road Edinburgh
 Evans Medical Supplies Ltd Speke Liverpool 19
 Gedon Richter (Great Britain) Ltd Waddington Road London N W 5
 Genatosan Ltd Loughborough Leicestershire
 Glaxo Laboratories Ltd Greenford Middlesex
 Imperial Chemical (Pharmaceuticals) Ltd Fulham Hall Wilmslow Manchester
 Lilly and Co Ltd Basinstoke Hants
 Menley and James Ltd 119-123 Coldharbour Lane London S E 5

Gonadyl Serie	Ampoules containing 400 or 1000 i.u.	Roussel
Luteoantin	Ampoules containing 100 or 400 units	Richter
Serogan	Ampoules containing 200 or 1000 i.u.	British Drug Houses

Posterior Lobe**Injection of Oxytocin**

(Injection of Oxytocin B.P.)	contains 10 units (oxytocic) per ml	
Oxytocin	0.5 and 1 ml ampoules and 5 ml vials	Parke Davis

Injection of Pituitary (Posterior Lobe)

(Injection of Pituitary (Posterior Lobe) B.P.)	contains 10 units (oxytocic) per ml	
Glandurtrin	0.5 and 1 ml ampoules and 10-ml vials	Richter
Infundibulin	0.5 and 1 ml ampoules and 10- and 25 ml vials	Evans
Infundin	0.5 and 1 ml ampoules	Burroughs Wellcome
Pitubulin	0.5 and 1 ml ampoules	Allen and Hanburys
Pituitary (Posterior Lobe) Extract	0.5 and 1 ml ampoules and 10- and 20-ml vials	Armour
	0.5 and 1 ml ampoules	Boots
	0.5 and 1 ml ampoules and 15 ml vials	British Drug Houses
	0.5 and 1 ml ampoules	Paines and Byrne
Pituitrin	0.5 and 1 ml ampoules and 10-ml vials	Parke Davis

Injection of Vasopressin

The B.P. injection contains 10 units per ml. The commercial preparation most nearly resembling it is the following:

Pitressin	0.5 and 1 ml ampoules containing 20 pressor units per ml	Parke Davis
-----------	--	-------------

Snuffs

Disipidin	100 gr. bottles or 14 gr. capsules	Paines and Byrne
Glandurtrin	500 i.u. per g.	Richter
Piton Powder	500 i.u. per g. in lactose	Organon
Pituitary (Posterior Lobe) Snuff	4 gr. capsules	Armour

Other Posterior Lobe Preparations

Pitoxilin	Extract of the posterior lobe supplied in 0.5 ml ampoules containing 5 units per ml, 0.5 and 1 ml ampoules and 10-ml bottles containing 10 units per ml, and 10 ml bottles containing 20 units per ml	Oxo
Pitressin Tannate in Oil	1 ml ampoules containing 5 pressor units	Parke Davis

THYROID**Antithyroid Substances**

Thiouracil	50, 100 or 200 mg tablets	{ British Drug Houses Imperial Chemical (Pharmaceuticals) Organon
	50- or 200-mg tablets	

(Tablets of Thiouracil B.P. contain 0.1 g. unless otherwise ordered.)

BIBLIOGRAPHY

GENERAL

- American Association for the Advancement of Science Chemistry and Physiology of the Hormones 1944 American Association for the Advancement of Science Washington
- American Medical Association Glandular Physiology and Therapy A symposium prepared under the auspices of the Council on Pharmacy and Chemistry of the American Medical Association 2nd Edition 1947 American Medical Association Chicago
- PEACH F A Hormones and Behaviour 1945 Harper New York
- BEAUMONT G E and DODD I C Recent Advances in Medicine 12th Edition 1947 Best C H and TAYLOR N The Physiological Basis of Medical Practice 4th Edition 1945 Baillière Tindall and Cox London
- FLRY J H Background to Therapeutics 1948 Oxford University Press London
- BURN J H Physiological Standardisation 1950 Oxford University Press London
- CAMEROY A T Recent Advances in Endocrinology 8th Edition 1947 Churchill London
- CANNON W B The Wisdom of the Body 2nd Edition 1939 Norton New York
- CANADIAN A P Clinical Endocrinology and Constitutional Medicine 1948 Muller London
- CLARK A J Applied Pharmacology 7th Edition 1940 Churchill London
- CRIBBER B L A Manual of Endocrine Therapy 1942 Macmillan London
- COBB I G The Glands of Destiny 3rd Edition 1947 Heinemann London
- CURRY A R Pharmacology and Therapeutics 14th Edition 1947 Revised by A GROLLMAN and D SALCHTER Churchill London
- EMMENS C W Principles of Biological Assay 1948 Chapman and Hall London
- PICARRA B J Diagnostic Synopsis of the Acute Surgical Abdomen 1949 Blackwell Scientific Publications Oxford
- FISHER L F and FIEBER M Natural Products related to Phenanthrene 3rd Edition 1949 Chapman and Hall London
- FULTON J F A Textbook of Physiology 18th Edition 1949 Section XI The Endocrine System by JANE A FISSELL Saunders London
- GADDUM J H Pharmacology 1st Edition 1948 Oxford University Press London
- GOLDBLATT H The Renal Origin of Hypertension 1948 Blackwell Scientific Publications Oxford
- GOLDBLATT H A Practical Endocrinology Symptoms and Treatment 2nd Edition 1955 Appleton Century London
- GOLDBLATT H A The Endocrine Glands 1939 Appleton Century London
- GOODMAN L and GILMAN A The Pharmacological Basis of Therapeutics A Textbook of Pharmacology Toxicology and Therapeutics for Physicians and Medical Students 1941 Macmillan London
- GREENBLATT R H Office Endocrinology 3rd Edition 1947 Thomas Springfield Illinois
- GREEN E K Editor The Practice of Endocrinology 1948 Eyre and Spottiswoode London
- GROLLMAN A Fertility and Endocrinology and Education 1947 Lippincott London
- HARRIS R S and THILLYN H V Editors Vitamins and Hormones Advances in Research and Applications Volumes I to VII 1943-1947 Academic Press New York
- HARROW H and SHERRIN C P The Chemistry of the Hormones 1934 Williams Wilkins Baltimore
- HOBGEN L T The Comparative Physiology of Internal Secretion 1927 Cambridge University Press London
- HOSKIN R G Endocrinology The Glands and Their Functions 1942 Routledge London
- KEMP W N Value of Hormones in General Practice 1940 Burgess Minneapolis
- LE MAPOLAND H S and TOZER F H W Endocrine Disorders in Childhood and Adolescence 1948 Hodder and Stoughton London
- MARTIN L and HYNES M Clinical Endocrinology 1949 Churchill London
- PRINCE G and THOMAS F A The Hormones Physiology Chemistry and Applications Volume I 1948 Academic Press New York

- Oppenheimer Son and Co Ltd Handforth Laboratories Clapham Road London S W 9
- Organon Laboratories Ltd Brettenham House Lancaster Place London W C 2
- Ortho Pharmaceuticals Ltd Lane End High Wycombe Buckinghamshire
- Oxo Ltd Thames House Queen Street Place London E C 4
- Faines and Byrne Ltd Pabym Laboratories Bilton Road Greenford Middlesex
- Parke Davis and Co Staines Road Hounslow Middlesex
- Pharmaceutical Laboratories Geigy Ltd National Buildings Parsonage Manchester 3
- Roussel Laboratories Ltd 4 Golden Square London W 1
- Wallace (Pharmaceutical Products) Ltd 198 Finchley Road London N W 3
- Wyeth and Bro Ltd Clifton House Euston Road London N W 1

BIBLIOGRAPHY

GENERAL

- American Association for the Advancement of Science Chemistry and Physics of the Hormones 1944 American Association for the Advancement of Science Washington
- American Medical Association Glandular Physiology and Therapy A symposium prepared under the auspices of the Council on Pharmacy and Chemistry of the American Medical Association 2nd Edition 1942 American Medical Association Chicago
- BEACH F A Hormones and Behaviour 1948 Harper New York
- BEAUMONT G F and DOUGLAS E G Recent Advances in Medicine 1st Edition 1947 Best C H and TAYLOR N The Physiological Basis of Medical Practice 4th Edition 1945 Baillière Tindall and Cox London
- BURN J H Background to Therapeutics 1948 Oxford University Press London
- BURN J H Biological Standardisation 1950 Oxford University Press London
- CAMERON A T Recent Advances in Endocrinology 6th Edition 1947 Churchill London
- CANNON W B The Wisdom of the Body 2nd Edition 1939 Norton New York
- CAWADIAS A P Clinical Endocrinology and Constitutional Medicine 1948 M. H. London
- CLARK A J Applied Pharmacology 7th Edition 1940 Churchill London
- CINBERG M I A Manual of Endocrine Therapy 1942 Macmillan London
- COBB I G The Glands of Destiny 3rd Edition 1947 Heinemann London
- CUSHNY A R Pharmacology and Therapeutics 13th Edition 1947 Revised by A. GROLLMAN and D. SLAUGHTER Churchill London
- EMERY C W Principles of Biological Assay 1948 Chapman and Hall London
- FIGARRA B J Diagnostic Synopses of the Acute Surgical Abdomen 1949 Blackwell Scientific Publications Oxford
- FIESER L F and FIESER M Natural Products related to Phenanthrene 3rd Edition 1949 Chapman and Hall London
- FULTON J F A Textbook of Physiology 16th Edition 1949 Section XI The Endocrine System by JANE A. RUSSELL Saunders London
- GADDUM J H Pharmacology 3rd Edition 1948 Oxford University Press London
- GOLDBLATT H The Renal Origin of Hypertension 1948 Blackwell Scientific Publications Oxford
- GOLDZIEHER M A Practical Endocrinology Symptoms and Treatment 2nd Edition 1935 Appleton Century London
- GOLDZIEHER M A The Endocrine Glands 1939 Appleton Century London
- GOODMAN L and GILMAN A The Pharmacological Basis of Therapeutics A Textbook of Pharmacology Toxicology and Therapeutics for Physicians and Medical Students 1941 Macmillan London
- GREENBLATT R B Office Endocrinology 3rd Edition 1947 Thomas Springfield Illinois
- GREEN E R Editor The Practice of Endocrinology 1948 Eyre and Spottiswood London
- GROLLMAN A Essentials of Endocrinology 2nd Edition 1947 Lippincott London
- HARRIS R S and J. HIGMAN K. V. Editors Vitamins and Hormones Advances in Research and Applications Volumes I to VII 1943-1949 Academic Press New York
- HARROW H and SHERWIN C P The Chemistry of the Hormones 1934 Williams Wilkins Baltimore
- HOBBS L T The Comparative Physiology of Internal Secretion 1927 Cambridge University Press London
- HOKINS R G Endocrinology The Glands and Their Functions 1942 Routledge London
- KEMP W A Valence of Hormones in General Practice 1949 Pergamon Minneapolis
- LE MARQUAND H S and TOZER F H W Endocrine Disorders in Childhood and Adolescence 1948 Hutter and Stoughton London
- MARTIN L and HYNES M Clinical Endocrinology 1948 Churchill London
- PINCUS C and THIMANN K. V. The Hormones Physiology Chemistry and Applications Volume I 1948 Academic Press New York

- RYNFARSON E H and GASTINEAU C F Obesity 1949 Blackwell Scientific Publications Oxford
- SELYE H *Textbook of Endocrinology* 2nd Edition 1949 Acta Endocrinologica Montreal
- SEVERINGHAUS E L Endocrine Therapy in General Practice 6th Edition 1947 Year Book Publishers Chicago
- SIMPSON S L Major Endocrine Disorders 2nd Edition 1948 Oxford University Press London
- STEPHENS G A Hormones and Vitamins 1947 Newnes London
- TURNER C D General Endocrinology 1948 Saunders London
- WALKER G F The Status of Enzymes and Hormones in Therapy 1935 Wright Bristol
- WERNER A A Endocrinology Clinical Applications and Treatment 2nd Edition 1942 Kimpton London
- WIGGERS C J Physiology in Health and Disease 5th Edition 1940 Kimpton London
- WOKES F A Textbook of Applied Biochemistry for Pharmacists and Pharmaceutical Students 1937 Bailière Tindall and Cox London
- WOLF W Endocrinology in Modern Practice 2nd Edition 1939 Saunders London
- WRIGHT S Applied Physiology 8th Edition 1945 Oxford University Press London
- ZONDEK H Diseases of the Endocrine Glands Translated by C F GILES 2nd English Edition 1944 Arnold London

ADRENALS

- ADDISON T On the Constitution and Local Effects of Disease of the Suprarenal Capsules 1855 Highley London
- BROSTER L R and others Adrenal Insufficiency and Intersexuality 1938 Chapman London
- BROSTER L R and VINES H W C The Adrenal Cortex A Surgical and Pathological Study 1933 Lewis London
- CANNON W B Bodily Changes in Pain Hunger Fear and Rage 2nd Edition 1929 Appleton Century London
- CANNON W B and ROSENBLUTH A Autonomic Neuro-effector Systems 1937 Macmillan London
- GROLLMAN A The Adrenals 1936 Williams Wilkins Baltimore
- HARTMAN F A and BROWNELL K A The Adrenal Gland 1949 Lea and Febiger Philadelphia
- RALLI A P Editor Adrenal Cortex 1950 Josiah Macy Jr Foundation
- ROWNTREE L G and SNELL A M A Clinical Study of Addison's Disease 1931 Saunders London
- SOFFER L J Diseases of the Adrenals 2nd Edition 1948 Kimpton London
- THORN G W and others The Diagnosis and Treatment of Adrenal Insufficiency 1949 Blackwell Scientific Publications Oxford

GONADS

- ALLEN F DANFORTH C H and DOBSON M A Editors Sex and Internal Secretion A Survey of Recent Research 2nd Edition 1939 Baillière Tindall and Cox London
- BISHOP P M G Gynaecological Endocrinology for the Practitioner 1946 Livingstone Edinburgh
- BOURNE A W Synopsis of Obstetrics and Gynaecology 10th Edition 1949 Wright Bristol
- BOURNE A W and WILLIAMS L Recent Advances in Obstetrics and Gynaecology 7th Edition 1947 Churchill London
- BOWES K Editor Modern Obstetrics and Gynaecology 1950 Putterworth London
- BREWS A Eden and Holland Manual of Obstetrics 9th Edition 1948 Churchill London
- BROSTER L R Endocrine Man A Study in the Surgery of Sex 1944 Heinemann London
- BURROWS H Biological Actions of Sex Hormones 1945 Cambridge University Press London

- BUXTON C L and ENGLE E T *Diagnosis and Therapy of Gynecological Disorders* 1949 Thomas Springfield Illinois
- COWIE A T *Pregnancy Diagnosis Tests A Review* 1948 Cultural Bureau Aberystwyth (Joint Publication No 13)
- FLUHMAN C F *Menstrual Disorders Pathology Diagnosis and Treatment* Saunders London
- HAMBLEY E C *Endocrinology of Woman* 1945 Thomas Springfield Illinois
- HARTMANN C C *The Time of Ovulation in Women* 1939 Baltimore
- HOFFMAN J *Female Endocrinology Including Sections on the Male* London
- HOBKINS R G *The Tides of Life The Endocrine Glands in Bodily Rhythms* Routledge London
- HOTCHKISS R S *Fertility in Men A Clinical Study of the Cause and Treatment of Impaired Fertility in Men* 1944 Lippincott London
- KERR J M M *Combined Textbook of Obstetrics and Gynecology for Medical Practitioners* 4th Edition 1944 Livingstone Edinburgh
- KOHL F C and SMITH P E *Sex Hormones Biological Symposia* V. Cattell New York
- KRUP I de *The Male Hormone* 1949 W H Allen London
- LANE ROBERTS C H SHARMAN A WALKER K and WIESNER B F *Impaired Fertility Pathogenesis Diagnosis and Treatment* Hamilton London
- LIPSCHUTZ A *The Internal Secretions of the Sex Glands* 1924 Williams Baltimore
- LOWESLEY O S SMITH D R and GUTIERREZ R *The Sexual Glands of the Male* 1942 Oxford University Press London
- National Committee on Maternal Health *Proceedings Conference on Diets for Sterility* January 26th and 27th 1945 at New York City Edited by E T Engle 1946 Thomas Springfield Illinois
- NOVAK E *Textbook of Gynecology* 3rd Edition 1949 Bailliere Tindall and Co London
- PARKES A S *The Internal Secretions of the Ovary* 1929 Longman London
- PEDERSEN BJERGAARD K *Comparative Studies concerning the Strengths of Steroidogenic Substances* 1939 Oxford University Press London
- PEEL J H *Textbook of Gynecology* 2nd Edition 1946 Heinemann London
- REYNOLDS S R M *Physiology of the Uterus With Clinical Correlations* 1933 Hoeber New York
- ROBSON J M *Recent Advances in Sex and Reproductive Physiology* 3rd Edition 1947 Churchill London
- SHAW W A *Textbook on Gynecology* 5th Edition 1948 Churchill London
- SIEGLER S L *Fertility in Women Causes Diagnosis and Treatment of Impaired Fertility* 1945 Heinemann London
- WALKER K and STRAUSS E B *Sexual Disorders in the Male* 3rd Edition 1949 Cassell London
- WHITE M M *The Symptomatic Diagnosis and Treatment of Gynecological Disorders* 2nd Edition 1946 Lewis London
- YOUNG J A *Textbook of Gynecology* 7th Edition 1947 Black London

PANCREAS

- BELL I T *Experimental Diabetes Mellitus* 1948 Thomas Springfield Illinois
- BEST C H *Diabetes and Insulin and the Lipotropic Factors* 1948 Thomas Springfield Illinois
- JENSEN H F *Insulin Its Chemistry and Physiology* 1938 Oxford University Press London
- JOSLIN E P Th *Treatment of Diabetes Mellitus* 8th Edition 1946 Lea and Febiger Philadelphia
- JOSLIN E P Th *Diabetic Manual for the Mutual Use of Doctor and Patient* 7th Edition 1941 Lea and Febiger Philadelphia
- LAWRENCE R D Th *Diabetic ABC A Practical Book for Patients and Nurses* 9th Edition 1946 Lewis London

- RYNEARSON E H and CASTINEAU C F Obesity 1949 Blackwell Scientific Publications Oxford
- SELYE H Textbook of Endocrinology 2nd Edition 1949 Acta Endocrinologica Montreal
- SEVERINGHAUS E L Endocrine Therapy in General Practice 6th Edition 1947 Year Book Publishers Chicago
- SIMPSON S L Major Endocrine Disorders 2nd Edition 1948 Oxford University Press London
- STEPHENS G A Hormones and Vitamins 1947 Nettes London
- TURNER C H General Endocrinology 1948 Saunders London
- WALKER G F The Status of Enzymes and Hormones in Therapy 1935 Wright Bristol
- WERNER A A Endocrinology Clinical Applications and Treatment 2nd Edition 1942 Kimpton London
- WIGGERS C J Physiology in Health and Disease 5th Edition 1949 Kimpton London
- WOKES F A Textbook of Applied Biochemistry for Pharmacists and Pharmaceutical Students 1937 Baillière Tindall and Cox London
- WOLF W Endocrinology in Modern Practice 2nd Edition 1939 Saunders London
- WRIGHT E Applied Physiology 8th Edition 1945 Oxford University Press London
- ZONDEK H Diseases of the Endocrine Glands Translated by C P GILES 2nd English Edition 1944 Arnold London

ADRENALS

- ADDISON T On the Constitution and Local Effects of Disease of the Supra renal Capsules 1855 Highley London
- BROSTER L R and others Adrenal Insufficiency and Intersexuality 1938 Chapman London
- BROSTER L R and VINES H W C The Adrenal Cortex A Surgical and Pathological Study 1933 Lewis London
- CANNON W B Bodily Changes in Pain Hunger Fear and Rage 2nd Edition 1979 Appleton Century London
- CANNON W B and ROSENBLUTH A Autonomic Neuro effector Systems 1937 Macmillan London
- GROLLMAN A The Adrenals 1936 Williams Wilkins Baltimore
- HARTMAN F A and BROWNELL K A The Adrenal Gland 1949 Lea and Febiger Philadelphia
- RALLI A P Editor Adrenal Cortex 1950 Josiah Macy Jr Foundation
- ROWNTREE L G and SNELL A M A Clinical Study of Addison's Disease 1931 Saunders London
- SOFFER L J Diseases of the Adrenals 2nd Edition 1948 Kimpton London
- THORN G W and others The Diagnosis and Treatment of Adrenal Insufficiency 1949 Blackwell Scientific Publications Oxford

GONADS

- ALLEN E DANFORTH C H and DOISY M A Editors Sex and Internal Secretions A Survey of Recent Research 2nd Edition 1939 Baillière Tindall and Cox London
- BISHOP P M F Gynaecological Endocrinology for the Practitioner 1946 Livingstone Edinburgh
- BOURNE A W Synopsis of Obstetrics and Gynaecology 10th Edition 1940 Wright Bristol
- BOURNE A W and WILLIAMS L Recent Advances in Obstetrics and Gynaecology 7th Edition 1947 Churchill London
- BOWES K Editor Modern Obstetrics and Gynaecology 1950 Butterworth London
- BREWS A Eden and Holland's Manual of Obstetrics 9th Edition 1948 Churchill London
- BROSTER I R Endocrine Man A Study in the Surgery of Sex 1944 Heinemann London
- BURROWS H Biological Actions of Sex Hormones 1945 Cambridge University Press London

- BUXTON C L and EVGLE E T *Diagnosis and Therapy of Gynecological Endocrine Disorders* 1949 Thomas Springfield Illinois
- COWIE A T *Pregnancy Diagnosis Tests A Review* 1948 Commonwealth Agricultural Bureaux Aberystwyth (Joint Publication No 13)
- FLUHMAN C F *Menstrual Disorders Pathology Diagnosis and Treatment* 1939 Saunders London
- HAMBLEY E C *Endocrinology of Woman* 1945 Thomas Springfield Illinois
- HARTMANN C G *The Time of Ovulation in Women* 1936 Williams Wilkins Baltimore
- HOFFMAN J *Female Endocrinology Including Sections on the Male* 1944 Saunders London
- HOSKINS R G *The Tides of Life The Endocrine Glands in Bodily Adjustment* 1933 Routledge London
- HORTCHKESS H S *Fertility in Men A Clinical Study of the Causes Diagnosis and Treatment of Impaired Fertility in Men* 1944 Lippincott London
- KERR J M M *Combined Textbook of Obstetrics and Gynecology for Students and Medical Practitioners* 4th Edition 1944 Livingstone Edinburgh
- KOCH F C and SMITH P E *Sex Hormones Biological Symposia Volume IX* 1942 Cattell New York
- KRUTY P de *The Male Hormone* 1949 W B Allen London
- LANE ROBERTS C S SHARLAN A WALKER K and WIESNER H P *Sterility and Impaired Fertility Pathogenesis Diagnosis and Treatment* 1939 Hamish Hamilton London
- LIPSCHUTZ A *The Internal Secretions of the Sex Glands* 1924 Williams Wilkins Baltimore
- LOWSELEY O S SMITH D R and GUTIERREZ R *The Sexual Glands of the Male* 1942 Oxford University Press London
- National Committee on Maternal Health *Proceedings Conference on Diagnosis in Sterility* January 26th and 27th 1945 at New York City Edited by E T ENGLE 1946 Thomas Springfield Illinois
- NOVAK E *Textbook of Gynecology* 3rd Edition 1949 Baillière Tindall and Cox London
- PARKES A H *The Internal Secretions of the Ovary* 1919 Longmans London
- PEDERSEN BJERGAARD K *Comparative Studies concerning the Strengths of Oestrogenic Substances* 1939 Oxford University Press London
- PEEL J H *Textbook of Gynecology* 2nd Edition 1946 Heinemann London
- REYNOLDS S R M *Physiology of the Uterus With Clinical Correlations* 1939 Hoeber New York
- ROBSON J M *Recent Advances in Sex and Reproductive Physiology* 3rd Edition 1947 Churchill London
- SHAW W A *Textbook on Gynecology* 5th Edition 1948 Churchill London
- SIEGLER S L *Fertility in Women Causes Diagnosis and Treatment of Impaired Fertility* 1945 Heinemann London
- WALKER K and STRALS E B *Sexual Disorders in the Male* 3rd Edition 1949 Cassell London
- WHITE M M *The Symptomatic Diagnosis and Treatment of Gynecological Disorders* 2nd Edition 1946 Lewis London
- YOUNG J A *Textbook of Gynecology* 7th Edition 1947 Black London

PANCREAS

- BELL E T *Experimental Diabetes Mellitus* 1948 Thomas Springfield Illinois
- BEST C H *Diabetes and Insulin and the Lapotrophic Factors* 1948 Thomas Springfield Illinois
- JENSEN H F *Insulin Its Chemistry and Physiology* 1938 Oxford University Press London
- JOSLIN E P *The Treatment of Diabetes Mellitus* 8th Edition 1946 Lea and Febiger Philadelphia
- JOSLIN E P *Diabetic Manual for the Mutual Use of Doctor and Patient* 7th Edition 1941 Lea and Febiger Philadelphia
- LAWRENCE R H *The Diabetic ABC A Practical Book for Patients and Nurses* 9th Edition 1946 Lewis London

LAWRENCE R. H. *The Diabetic Life Its Control by Diet and Insulin* 14th Edition 1950 Churchill London

PARATHYROID

- ALBRIGHT F. and REIFENSTEIN E. C. Jr. *The Parathyroid Glands and Metabolic Bone Disease* 1948 Baillière Tindall and Cox London
- CANTAROW A. *Calcium Metabolism and Calcium Therapy* 2nd Edition 1933 Lea and Febiger Philadelphia
- GILMOUR J. R. *The Parathyroid Glands and Skeleton in Renal Disease* 1948 Oxford University Press London
- HARRIS H. A. *Bone Growth in Health and Disease* 1933 Oxford University Press London
- HESSE A. T. *Rickets Including Osteomalacia and Tetany* 1929 Lea and Febiger Philadelphia
- SHIELLING D. H. *The Parathyroids in Health and Disease* 1935 Lea and Febiger Philadelphia

PITUITARY

- Association of Research in Nervous and Mental Diseases. *The Pituitary Gland* 1938 Williams Wilkins Baltimore
- BISHOP P. M. F. *Gynaecological Endocrinology for the Practitioner* 1946 Livingstone Edinburgh
- BOURNE A. W. *Synopsis of Obstetrics and Gynaecology* 10th Edition 1949 Wright Bristol
- BOURNE A. W. and WILLIAMS L. *Recent Advances in Obstetrics and Gynaecology* 7th Edition 1947 Churchill London
- BREWS A. *Eden and Holland's Manual of Obstetrics* 9th Edition 1948 Churchill London
- BUXTON C. L. and ENGLE E. T. *Diagnosis and Therapy of Gynaecological Endocrine Disorders* 1949 Thomas Springfield Illinois
- VAN DYKE E. B. *The Physiology and Pharmacology of the Pituitary Body* Volume I 1936 Volume II 1939 University of Chicago Press Chicago
- FISHER C. INGRAM W. R. and RAYSON S. W. *Diabetes Insipidus and Neuro* Hormonal Control of Water Balance 1938 Edwards Brothers Ann Arbor Michigan
- HAMBLETON E. C. *Endocrinology of Woman* 1945 Thomas Springfield Illinois
- HARTMANN C. G. *The Time of Ovulation in Women* 1936 Williams Wilkins Baltimore
- HOFFMAN J. *Female Endocrinology Including Sections on the Male* 1944 Saunders London
- HOTCHKISS R. S. *Fertility in Men A Clinical Study of the Causes Diagnosis and Treatment of Impaired Fertility in Men* 1944 Lippincott London
- KAPLAN J. M. M. *Combined Textbook of Obstetrics and Gynaecology for Students and Medical Practitioners* 4th Edition 1944 Livingstone Edinburgh
- LANE ROBERTS C. S. SHARMAN A. WALKER K. and WIESNER B. P. *Sterility and Impaired Fertility Pathogenesis Diagnosis and Treatment* 1939 Hamish Hamilton London
- National Committee on Maternal Health. *Proceedings Conference on Diagnosis in Sterility* January 26th and 27th 1945 at New York City Edited by E. T. ENGLE 1946 Thomas Springfield Illinois
- NOVAK E. *Textbook of Gynaecology* 3rd Edition 1949 Baillière Tindall and Cox London
- PEEL J. H. *Textbook of Gynaecology* 2nd Edition 1946 Hermann London
- ROBSON J. M. *Recent Advances in Sex and Reproductive Physiology* 3rd Edition 1947 Churchill London
- SHAW W. A. *Textbook of Gynaecology* 5th Edition 1948 Churchill London
- SIEGLER S. L. *Fertility in Women Causes Diagnosis and Treatment of Impaired Fertility* 1945 Hermann London
- WALKER K. and STRAUSS E. B. *Sexual Disorders in the Male* 3rd Edition 1949 Cassell London
- WHITE M. M. *The Symptomatic Diagnosis and Treatment of Gynaecological Disorders* 2nd Edition 1946 Lewis London
- YOUNG J. A. *Textbook of Gynaecology* 7th Edition 1947 Black London

THYROID

- CRILE G. Jr. *Practical Aspects of Thyroid Disease* 1949 Saunders London
- HARRINGTON C. R. *The Thyroid Gland* 1933 Oxford University Press London
- JOLL C. A. *Diseases of the Thyroid Glands with Special Reference to Thyrotoxicosis* 1937 Heinemann London
- MCCARRISON R. *The Pathology of Endemic Goitre* 1915 Bale London
- MCCLENDON J. F. *Iodine and the Incidence of Goitre* 1939 Oxford University Press London
- MCEWAN P. *The Clinical Picture of Thyrotoxicosis* 1948 Oliver Edinburgh
- MEANS J. H. and others. *The Thyroid and its Diseases* 2nd Edition (1941) Lippincott London

INDEX

- ABORTION progestogen treatment of 169
- Acetoacetic acid 21 25
- Acetone 21 25
- 3(β) Acetoxy Δ^4 isonoretholenic acid 64
- 3(β) Acetoxy nor 5 alloetholenic acid ■
- Acidophil cells 39
- Acromegaly 30 152 153
- ACTH *see* Adrenocorticotrophic hormone
- Addison's disease causes of 37 160
 - treatment of 161
- Adrenal cortex commercial preparations of 194
 - physiology of 37
 - relationship to other glands 37 160
- Adrenal cortical deficiency 37 160
 - extracts action and uses of 37 160
 - hormones chemistry of 77
 - isolation of 77
 - standardisation of 127
- Adrenal medulla 34
- Adrenal virilism 37
- Adrenalin 34
- Adrenalin and Chloretone preparations 193
- Adrenalin Chloride Solution 192
- Adrenalin and Cocaine Hypodermic Tablets 194
- Adrenalin Hypodermic Tablets 194
- Adrenalin Inhalant 192
- Adrenalin in Oil 192
- Adrenalin Ointment 193
- Adrenalina 184
- Adrenaline action and uses of 35 159
 - assay of 129
 - chemistry of 57
 - commercial preparations 192
 - in dentistry 160
 - eye lotion 185
 - ointment formula 185
 - inhalants 192
 - Injection of 160 184 192
 - isolation of 56
 - nasal sprays 185
 - ointments 185 193
 - pharmacy of 184
 - suppositories 185 193
 - Suspension with Ascorbic Acid 192
 - synthesis of 57
- Adrenaline ascorbate 160 185
- Adrenaline Borate Solution 193
- Adrenaline Chloride Solution 192
- Adrenaline Hydrochloride Solution of 160 184
- Adrenaline mucate injection formula 184
- Adrenaline Tartrate Injection of 184
 - Spray Solution 192
- Adrenalinum 184
- Adrenals bibliography of 208
 - chemistry of 56
 - commercial preparations of 192
 - early work on 5
 - physiology of 34
- Adrenocorticotrophic hormone action and uses of 134
 - assay of 122
 - commercial preparation 202
 - function of 29 33 38
 - pharmacy of 170
 - in rheumatism 155 162
- Adrenosterone chemistry of 79
 - from synthesis of cortisone 97
- Adrenutol 192
- Adrephine preparations 19. 193
- Allen Dossy test 133
- Allergy to hormones 172
- Alloran diabetes 27
- Ambion I and II 203
- Amenorone 196
- Amenorrhoea treatment of 157
- Androgens action and uses of 170
 - administration of 170
 - chemistry of 74
 - commercial preparations 194
 - human excretion of 106
 - isolation of 74
 - pharmacy of 186
 - standardisation of 134
 - unit of 134
- Androstane 75
- Androstandione 75
- Androstendione 76
- Androsterone chemistry of 70 75
 - derivation of 37
 - isolation of 74
 - synthesis of 84
 - unit of 134
 - in urine 107
- Anhyd obdrotypovestosterone 103
- Ancestrus 42
- Anol 100
- Anteplian 203
- Anterior lobe *see* Pituitary anterior lobe
- Anterior Pituitary Extract 203
- Antidiuretic activity assay of 126
- Antihormones 138
- Anti tetany 10 18
- Antihypoid substances 15 141 183 204
- Anto tab 203
- Antoxylin preparations 203
- Antrodin 203

- Antuitrin S 203
 Antuitrin Growth 202
 Anusan 193
 Aqueous Solution of Iodine 14
 Arden iodinated 15
 Armo-Nestrol preparations 196
 Arterenal 58
 Artificial hormones chemistry of 99
 Artificial estrogens *see* Estrogens
 artificial
 Aschheim Zondek test 46
 Assay biological limits of error in 17
 methods of computation of 115
 relative accuracy of methods of 135
 requirements for 113
 Asthma 160
 AT10 18 144 202
 Auer's Slutz Rule ■
 Avolotl 13

 BASAL metabolic rate determination of 14
 uses of 13
 Basedow's disease 10
 Barbexstrol 196
 Basophil cells 30
 Benedict's qualitative test 24
 quantitative test 24
 reagent 24
 Benedict-Roth apparatus 14
 Benzo-Gynestryl 198
 Benztrone 198
 Bibliography 207
 Bile acids chemistry of 60 64
 (—) Bisdehydrodihydroxylic acid 104
 Bisdehydrodihydroxylic acid synthesis of 105
 Blood sugar determination 25
 levels 21
 vitamins and 149
 Botowenn 99
 Breast cancer treatment of 169 172
 engorgement prevention of 167
 β -Bromo- β -phenyl α -di-*p*-
 ethoxyphenyl ethylene 102

 CALCIFEROL action and uses of 17 18
 in tetany 145
 Calcium gluconate 18
 Calcium metabolism 17
 Calcium serum 17
 Cancer of the breast 169 172
 estrogens and 168
 see also Carcinoma
 Carbohydrate metabolism 20
 Carcinoma prostate 168
 Cardiac glycosides 69
 chemistry of 66
 Casein iodinated 15
 Castration effects of 40
 physiological 164
 Cholan c acid 64
 Cholestanol 67
 Cholestenone 62
 Cholesterol 62
 Cholic acid 64
 Chorionepithelioma 47
 Chorionic gonadotrophin action and
 uses of 156
 assay of 123
 commercial preparations 203
 Injection of 180
 pharmacy of 180
 physiology of 31
 unit of 123
 Chromophil cells 30
 Chromophobe cells 30
 Chvostek's sign 144
 Collyrium Adrenalinae Compositum 185
 Coma diabetic (hyperglycemic) 21 150
 Commercial hormone preparations 192
 Compound E 78
 Coprostanol 62
 Coprosterol 62
 Corpus luteum commercial preparations
 of 200
 development of 42 44
 Cortical extracts 194
 Corticosterone chemistry of 78
 synthesis of 94
 Corticotrophic hormone *see* Adreno-
 corticotrophic hormone
 Cortigen 194
 Cortin 161
 Cortisol 194
 Cortisone in adrenal crises 161
 chemistry of 78
 diabetogen action of 26
 injection of 188
 in rheumatoid arthritis and rheu-
 matic fever 38 162
 synthesis of 97
 Cortisone acetate 97
 Cortrophin 20
 Cowper's glands 39
 Cretinism causes of 10 140
 treatment of 140
 Cryptorchidism treatment of 158
 Crystalline insulin 53
 Cushing's syndrome 38 152 161

 D.B.E. chemistry of 102
 clinical potency of 165
 Dehydro- α -androsterone chemistry of 70
 75
 synthesis of 86
 in urine 107
 Dihydrocort- α -sterone chemistry of 78
 synthesis of 96
 Dihydrodihydroxylic acid 164
 Delay Insulin A.B. 202
 Delayed action insulin assay of 132
 Deoxycholic acid 64 96 98
 Deoxycort- α -sterone *see* Deoxycortone
 Deoxycortone assay of 127
 chemistry of 78
 synthesis of 95

- Deoxycortone acetate action and uses of 37 161
 commercial preparations 194
 implantation of 161
 tablets 194
 Injection of 194
 percutaneous administration of 187
 pharmacy of 188
 in rheumatism 162
 in shock 163
 sublingual administration of 186
 tablets 194
 synthesis of 35
- Desoxycorticosterone *see* Deoxycortone
- Diabetes alloxan 27
- Diabetes innocens 21
- Diabetes insipidus physiology of 33
 treatment of 159
- Diabetes mellitus 21 147 176
 diagnostic test for 22
 mechanism of 146
- Diabetes phloridzin 27
- Diabetes tennifluis 159
- Diabetic coma 21 150
- Diabetogenic substance 29
- Diels hydrocarbon 66
- Diencetrol chemistry of 100
 commercial preparations 196
 cream 186
 potency of 164
 synthesis of 101
- Diethylstilbestrol *see* Stilbestrol
- Digitalis glycosides 66
- Digitogenin 66
- Digitoxigenin 66
- Digitoxin 66
- Digitoxose 66
- Digoxigenin 66
- Digoxin 66
- Dihydrotachysterol 18 202
 in tetany 144
- 4 4 Dihydroxydibenzyl 100
- 4 4 Dihydroxydiethyl stilbene 99
- 4 4 Dihydroxydiphenyl 99
- 4 4 Dihydroxy $\gamma\delta$ diphenylhexadiene 100
- 4 4 Dihydroxy $\gamma\delta$ -d phenyl α hexane 100
- 4 4 Dihydroxydiphenylmethane 99
- 4 4 Dihydroxystilbene 99
- pl. 3 5 Duodotthyronine 52
- Diiodotyrosine tablets 205
- 3 5 Duodotthyrosine 52
- Dimenformin 198
- Dimenformon Dipropionate 197
- Diœstrus 42
- Diosgenin 66 93
- Dipron 198 201
- Disipidin 204
- D O C A 194
- Doisynolic acid chemistry of 104
- Dry Thyroid 183
- Ductless glands 1
- Du arfism causes of 30 140 152
 treatment of 141 153
- Dysmenorrhœa 170
- Dystrophia adiposa genitalis 152
- FLITYRAN tablets 205
- Enclosed vessel assay technique 130
- Endemic goitre 133
- Endocrine glands 1
- Enseals Diethylstilbestrol 200
- Ephetonogen preparations 193
- Epinephrine origin of 34
 pharmacy of 184
 solution 184
- Equilenin isolation of 72
 synthesis of 82
- (+) Equilenin chemistry of 70 74
 isolation of 72
- Equilin chemistry of 70 74
 isolation of 72
- Erb's sign 144
- Ergostanol 64
- Ergosterol 62 63
- Ergon S preparations 195
- Eschatin 194
- Estigyn preparations 196
- Ethidol 196
- Ethinylœstradiol action and uses of 164
 commercial preparations 196
 synthesis of 103
 Tablets of 196
- Ethinyl Estryl 196
- Ethisterone commercial preparations 201
 dosage and uses of 160
 sublingual administration of 186
 synthesis of 103
- Eucyclin preparations 196
- Eucortone 194
- Eunuchism 170
- Eunuchoidism treatment of 158 170
- Excret on products 106
- Exophthalmic goitre 10
- Exophthalmos 10
- Extractum Pituitarii Liquidum 181
- F S H 31
- Fallopian tubes 41
- Fermentation test for sugar in urine 24
- Ferric chloride (Gerhardt's) test 25
- Folin and Wu method 25
- Follicle stimulating hormone 29 31
- Friedman test 46
- Fröhlich's syndrome cause of 152
 treatment of 158
- Fructosuria 147
- Fucosterol 11
- Functional hemorrhage 172
- GERHARDT'S test 25
- Gestone 201
- Gestone Oral 201
- Gestyl 203

- Giantism 151 153
 Grand Reagent T 71
 Groggenin 66
 Gtrogenin 66
 Gtrogen 66
 Glands ductless or endocrine 1
 Glanduatum 203
 Glandubolin 197
 Glanducorp n 201
 Glandu trin 204
 Glob n Insulin (with Zinc) 202
 Globin Zinc Insulin action and uses of 147 148
 assay of 137
 duration of effect of 23
 Injection of 178 202
 pharmacy of 178
 Glosso Sterandryl 195
 Glucose tolerance test 22
 Glycogen 20
 Glycogenate 20
 Glycosuria renal 21 147
 Glycotrophic substance 20
 Goutre endemic or simple 130
 exophthalmic 10
 toxic 10
 Gonad preparations standardization of 137
 Gonadotraphon F S 11 203
 Gonadotraphon L H 203
 Gonadotrophic activity assay of 123
 Gonadotrophic hormones 29 179
 Gonadotroph n chorionic action and uses of 156 157
 assay of 123
 commercial preparations 203
 Injection of 180
 physiology of 31
 unit of 123
 Gonadotrophin serum action and uses of 156 157
 assay of 125
 commercial preparations 203
 Injection of 180
 physiology of 31
 unit of 125
 Gonadotrophins assay of pituitary 123
 Gonadotroph num Chor on cum 156 180
 Gonadotrophinum Sericum 156 180
 Gonad anterior lobe relationship to 30
 bibliography of 708
 early work on 7
 physiology of 35
 Gonady 1 Chorionic 703
 Gonady 1 Seric 204
 Gonan 203
 Gonorrheal vulvovaginitis treatment of 168
 Graafian follicle 43
 Graaf disease 10
 Growth hormone action of 153
 assay of 121
 commercial preparation 702
 Growth hormone function of 29
 pharmacy of 179
 physiology of 30
 Gull's disease 141
 Guerin test 47
 Gynformon preparation 148
 Gynosteryl implants 197
 Gynolurine preparation 201
 Gnoson 200
 HEMORRHAGE treatment of excessive 167
 Hagedorn acid Jensen method 10
 Hay fever adrenaline treatment of 160
 Heutal 197
 Hæstrol 100 164
 commercial preparations 197
 synthesis of 101
 Hæstrol dipropionate 164
 Injection 197
 Hogben's pregnancy test 12 47
 Hormone all right at sets 188
 Hornones artificial chemistry of 80
 Houssay animals 28
 Hydatisform mole 47
 3 Hydroxycholesterol 17 one 107
 β Hydroxybutyric acid 21
 3(s) Hydroxycholesterol acid 64
 17 Hydroxy 11 dehydrocorticosterone 78
 21 Hydroxypregnosterone 78
 p Hydroxypropenylbenz 100
 Hydroxycholesterol acid 64
 Hypercorticalism 160
 Hyperdure Adrenaline 193
 Hypoglycemic coma 150
 Hyperparathyroidism 17
 treatment of 143
 Hypothyroidism 10
 treatment of 141
 Hypoglycemia 149
 Hypogonadism primary 170
 Hypoparathyroidism 147
 Hypoparathyroidism diagnosis of 144
 effects of 143
 treatment of 144
 Hypophysis 28 55
 Hypopituitarism 157
 Hypothalamic syndrome 33
 Hypothyroidism 10 140
 IDENTIFICATION of insulin packs 178
 Implantation of deoxycortone acetate pellets 161 188
 Infantile myxedema 140
 Infundibulin 201
 Infundin 204
 Injection of insulin 160 184
 Injection of Deoxycortone Acetate 188
 Injection of Gonadotrophin Chorionic 180
 Injection of Gonadotrophin Series 180
 Injection of insulin 147 177

- Injectio Insulini Globuli cum Zinco 178
 Injectio Insulini Irotaminati cum Zinco 147 177
 Injectio Estradioli Dipropionatis 188
 Injectio Estradioli Monobenzoatis 164 188
 Injectio Oxytocini 158 181
 Injectio Pituitarii Posterioris 158 181
 Injectio Progesteroni 169 188
 Injectio Suprarenali Corticis 161
 Injectio Testosteroni Propionatis 170 188
 Injectio Vasopressini 158 182
 Insulin A B Delay 202
 Insulin action of 21 146
 assay of 131
 chemistry of 53
 crystalline 53
 delayed action assay of 132
 dosage of 148
 globin zinc 23 147 178 202
 injection of 147 177 202
 isolation of 53
 legal requirements for 189
 local application of 179
 in non diabetic conditions 150
 ointment 179 202
 ordinary 147 177
 packs identification of 178
 pharmacy of 176 177
 preparations 22 147
 action of 147
 commercial 202
 preservation of 178
 protamine 23
 protamine zinc 23 147 177 202
 regular 147 177
 in schizophrenia 150
 secretion of 20
 soluble 147 177
 standard preparation of 131
 tablets 179 189
 unit of 131
 unmodified 147 177
 Insulinum 177
 International standards 111
 International units 112
 Iodinated arden assay of 130
 use of 15 130
 Iodinated casein assay of 130
 use of 15 130
 Iodinated plasma assay of 130
 use of 15
 Iodinated proteins 15
 Iodine Aqueous Solution of 14
 in goitre 139
 radioactive 14 143
 Iodothyroglobulin 50 52
 Islets of Langerhans 20 53

 KENDALL'S Compound E 78
 Ketodestrin 198
 Ketogenic substance 29
 Ketohydroxyoestrin 72
 Ketone bodies 21
 in urine tests for 23
 17 Keto steroids in diagnosis use of 160
 in urine 107
 1 Keto 1 2 3 4 tetrahydrophenanthrene 99
 Kolpon vaginal bougies 199
 Kraurosis 167

 LACTATION inhibition of 164 167 172
 Lactogenic hormone action of 154
 assay of 120
 pharmacy of 179
 physiology of 29 33
 standard preparation of 120
 unit of 120
 Legal requirements for hormones and their preparations 188
 L.H. 31
 Limits of error computation of 115 117
 Lipocair 28
 Liquor Adrenalinae Hydrochloridi 160 184
 Liquor folliculi 44
 Liquor Thyroides 183 205
 Lithocholic acid 64
 Lugol's solution 14
 Lutein 44
 Luteinizing hormone 29 31
 Luteoantun 204
 Luteomensin 201
 Luteotrophic hormone 29 33 154 179
 Lutocyclin preparations 201
 Lutogyl preparations 201
 Lutren 201
 Lynoral 196

 MALE reproductive system 39
 Male toad tests 47
 Mammary carcinoma 169 172
 Manufacturers of hormone preparations 205
 Marrianoic acid chemistry of 104
 Mastitis 172
 Melanophores 34
 Menformon 198 199
 Menopausal disorders treatment of 167
 Menorrhagia 167 170
 Menstrual cycle 43
 3 Methyl 1 2 cyclopentenophenanthrene 66
 Methyltestosterone commercial preparations 194
 sublingual administration of 186
 synthesis of 103
 tablets 195
 uses of 170
 Methylthiouracil action of 15
 in hyperthyroidism 141 183
 Tablets of 205
 Meteorus 42
 Metropatha hemorrhagica 167 170

- Mexican salamander 13
 Mircyston methyltestosterone 194
 estrone 198
 progesterone 207
 Mouse convulsion method 131
 Mucous edema 141
 Myxoedema 10 140
 infantile treatment of 140
 treatment of 141

 NEBULA Adrenalinae 185
 Nebula Adrenalinae et Atropinae Composita 185
 Neo-Hombrol preparations 190 196
 Nitroprusside test 25
 Noradrenaline action of 36
 chemistry of 58

 Obesity thyroid in 143
 Edema mucous 141
 a Estradiol chemistry of 70 73
 b Estradiol 73
 Estradiol action and uses of 41 164
 chemistry of 73
 commercial preparations 197
 secretion of 31 41
 synthesis of 80
 ointment 187 197
 Estradiol d propionate chemistry of 73
 injections of 197
 Estradiol monobenzoate chemistry of 73
 commercial preparations 198
 injections 184 188 198
 standard preparation of 132
 unit of 132
 Estrin 198 199
 Estrin capsules 198
 chemistry of 70 73
 isolation of 71
 synthesis of 80
 Estroform preparations 198 199
 Estrogen action and uses of 164
 administration of 165 186
 artificial actions and uses of 164
 chemistry of 99
 and cancer 168
 clinical applications of 168
 commercial preparations 196
 dosage of various 166
 isolation of 71
 in mammary carcinoma 169
 natural 164
 chemistry of 69
 pharmacology of 186
 in prostatic carcinoma 168
 standardization of 132
 synthetic *see* Estrogens artificial
 toxicity of 166
 Estrogen action and uses of 164 167
 administration of 187
 chemistry of 72
 commercial preparation 198 199
 Estrone excretion of 106
 isolation of 71
 nasal solution of 187
 pellets 187 192
 standard preparation of 132
 synthesis of 80
 tablets 186 199
 unit of 132
 (+) Estrone 70 72
 Estrovalve 199
 Estrus 42
 Oleum ketodestatin 199
 Oraluton 201
 Orasecton 196
 Ora iron 195
 Otitis fibrosa cystica 145
 Ovaries cyclic changes in 31 44
 function of 31 38 41
 O endosyn 200
 Ovocyclin preparations 197 198
 Oxytocic activity assay of 125
 Oxytocin action and uses of 33 158
 injection of 158 181 204
 pharmacology of 181

 PABESTROL preparations 199 200
 Fabestrol 199
 Pancrea bibliography of 209
 chemistry of 53
 collaborative work on 6
 relationship to other endocrine glands 26
 pharmacology of 176
 physiology of 19
 Pancreatrophic substance 26 29
 Paneston 195
 Parathormone 207
 Parathyroid hormone assay of 130
 commercial preparations 207
 and tetany 16
 Parathyroid injection 176
 assay of 130
 uses of 144
 Parathyroid unit 130
 Parathyroidectomy effects of 16
 Parathyroids action and uses of 143
 bibliography of 210
 chemistry of 53
 description of 15
 discovery of active principle of 6
 pharmacology of 176
 physiology of 15
 standardization of extracts of 130
 Parathyrotrophic hormone or substance 16 9
 Parodol 202
 Parom 205
 Parry's disease 19
 Pars intermedia 33
see Pentenophanthrene 50
 Ictenour 147
 Peptic ulcer prophylactic treatment of 159

- Perandren commercial preparations 19
 196
 Percorten commercial preparations 194
 Pharmacy and Poisons Act 1933 183
 Phloridzin diabetes 27
 Phosphorus metabolism 17
 Physiological castration 164
 Physostig 203
 Phytosterols 62
 Pitalin 193
 Pitecan Snuff 203
 Pitubulin 204
 Pitocin 204
 Piton Powder 204
 Pitoxilin 204
 Pitrenal n 193
 Pitressin commercial preparations 204
 Pituitary ba ophilism 152 161
 bibliography of 210
 cachexia 152
 chemistry of 55
 commercial preparations 202
 extract 181
 gonadotrophins action and uses of
 156
 assay of 123
 early work on 7
 legal requirements for 189
 physiology of 28
 snuffs 204
 Pituitary anterior lobe action and uses
 of 151
 chemistry of 56
 commercial preparations 202 203
 hormones of 29
 physiology of 29
 relationship to gonads 30
 standardisation of hormones of 119
 structure of 30
 Pituitary posterior lobe chemistry of 55
 commercial preparations 204
 emulsion 182
 extracts 181 204
 action and uses of 158
 standardisation of 125
 Injection 181 204
 action and uses of 158
 legal requirements 189
 nasal jelly 182
 pharmacy of 182
 physiology of 33
 powder 182
 uses of 159
 unit of 125
 Pituitrin 204
 Posterior lobe *see* Pituitary posterior
 lobe
 Potency computation of 115
 methods of assaying relative 118
 Pregnancy diagnosis 46
 Pregnanediol 46 106
 glucuronide 106
 Pregnenolone 103
 Pregnyl 203
 Pressor activity assay of 127
 Progesterone action and uses of 169
 administration of 169
 assay of 133
 chemistry of 70 77
 commercial preparations 201
 excretion of 44 106
 function of 44
 implantation tablets 201
 Injection of 169 201
 isolation of 76
 production of 31 41
 standard preparation of 133
 synthesis of 89
 unit of 133
 Progestin 202
 Progestogens commercial preparations
 of 200
 Progestoral 201
 Progynon preparations 197 199 199
 Prolactin action and uses of 154
 assay of 120
 function of 29 33
 pharmacy of 179
 Prolan 203
 Proluton 202
 Pro-oestrus 42
 Propylthiouracil 15 141 183
 tablets 205
*cyclo*Propylthiouracil 142
 Prostate gland 39
 Prostatic carcinoma treated by oestrogens
 168
 hypertrophy 171
 Protamine inulin 23 55
 Protamine zinc insulin action of 73 148
 assay of 132
 commercial preparation 202
 Injection of 147 177 202
 pharmacy of 177
 Proteins iodinated 15 130

 QUANTAL responses 114

 RABBIT blood sugar method of assay 131
 Radioactive iodine action and uses of
 14 143
 Regular insulin 147 177
 Reproductive cycle in mammals 42
 system in the female 41
 in the male 39
 Rheumatic fever cortisone treatment of
 38
 Rheumatoid arthritis 38
 Rheumatism decortone acetate in
 162
 Rothera's test 25

 SAPOGENINS chemistry of 66
 Saponins 66
 Sapotalene 66
 Sarmenotegen n 99

- Sarsaparilla 66
 Sarsasapogenin 66 93
 Schizophrenia insulin treatment in 150
 Sedestrol 200
 Seminal vesicles 39
 Semiferous tubules 39
 Semile vaginitis 167
 Serogan 204
 Serum calcium 17 18
 Serum gonadotrophin action and uses of 31 156
 assay of 125
 commercial preparations 203
 Injection of 180
 pharmacology of 180
 unit of 125
 Serum sickness 160
 Sex glands physiology of 38
 Sex hormones chemistry of 69
 Simmonds disease 152 154
 Sitostanol 63
 β Sitostenone 63
 Sitosterol α β and γ 62 63
 Sodium Estrone Sulphate 164 199
 Sodium pregnandiol glycerone date 46
 Sol ketodestrin 199
 Solestrin 109
 Soluble insulin 147 177
 Solution of Adrenaline 193
 South African clawed toad 12
 Spermatozoa 39
 Standard preparations 111
 Standards international 111
 Sterandryl preparations 195 198
 Stereochemical configuration 61
 Stereochemistry of the steroids 67
 Sterility treatment of 158 171
 Steroid derivatives synthesis of 103
 Steroid hormones excretion products of 106
 synthesis of 79
 Steroid sapogenins 60
 Steroids stereochemistry of 60
 Sterols chemistry of 60 61
 isolation of 6
 stereochemistry of 67
 Stigmastanol 63
 Stigmastanol 62 63 64
 Stilboestrol action and uses of 164
 chemistry of 100
 commercial preparations 199
 Compound 200
 cream formula for 187
 and glycerol 27
 and lactic acid pessaries 199
 in mammary carcinoma 160
 nasal administration of 187
 in prostatic carcinoma 168
 synthesis of 100
 tablets 165 200
 Stilboestrol d propionate commercial preparations 200
 Stilbofax 200
 Strogene 194
 Strophanthus 66 99
 Sugar in blood estimations 25
 in urine qualitative tests for 24
 quantitative tests for 24
 Sulphaguanidine 15
 Suppositorium Adrenaline 185
 Suppository Adrenaline et Cocaine 185
 Supracort 194
 Suprarenal Cortex Extract 194
 Suprarenal cortex injection of 161
 Suprarenal gland physiology of 34
 substances legal requirements for 190
 Suprarenal 193 194
 Sycebreed 19
 Synapoid 203
 Synovial preparations 194
 Syntestrin 200
 Synthetic estrogens see Estrogens artificial
 TABLET Echisteron 186 201
 Tablet D enoestrol 186 198
 Tablet Hexoestrol 186 197
 Tablet Methyltestosterone 186 195
 Tablet Estrone 164 186 199
 Tablet Stilboestrol 186 199
 Tablet Thyro de 183
 Test method choosing an assay 115
 rate accuracy of assay 135
 Testes function of 38 39
 undescended androgen treatment of 171
 Testosterone action and uses of 170
 administration of 170
 assay of 134
 chemistry of 70 76
 commercial preparations 195
 excretion of 106
 secretion of 39
 Testosterone propionate administration of 170
 assay of 134
 commercial preparations 195 198
 Injection of 170 189 195
 Thiourea 15 141
 Thyranon 205
 Thyrocol 205
 Thyroglobulin Tablets 205
 Thyroid bibliography of 211
 chemistry of 50
 colloid 11
 commercial preparations 204
 in cretinism 140
 and diabetes mellitus 26
 Dry 183
 Extract 183
 Gland 183
 description of 9
 early work on 6
 substances legal requirements for 190

- Thyroid in gynaecology 143
 and metamorphosis 11
 microscopy 11
 in myxodema 141
 in obesity 143
 ointment 183
 pharmacy of 183
 physiology of 10
 preparations 143 204
 assay of 129
 relationship to other endocrine glands 13 26
 Tablets of 183 204
 uses of 10 139
- Thyroideum 183
- Thyroideum Siccum 183
- Thyronine 50
- Thyrotoxicosis primary 10
- Thyrotrophic hormone action of 32 155
 assay of 119
 function of 29
 packing of 179
 physiology of 11
- Thyroxine action of 13
 assay of 129
 chemistry of 50
 ointment 183
 pharmacy of 183
 production of 9
 tablets 205
 uses of 139 141
- DL* Thyroxine 51
- L* Thyroxine 50 52 183
- Thyroxine sodium 141 183 205
- L* Thyroxine sodium 141 205
- Thyroxinsodium 183
- Tigogenin 66
- Toxic goitre 10
- Tri *p* an sylbromoethylene 102
- Trillin 93
- 1 2 7 Trimethylnaphthalene 66
- Triphenylchloroethylene 165
- Triphenylethylene 102
 derivatives chemistry of 107
- Trousseau's sign 143
- L* Tyrosine 52
- UNDEP* preparations 198
- Undescended testes 171
- Units international 112
- Unmodified insulin 147 177 202
- Urine 17 keto steroids in 107
 ketone bodies in tests for 25
 sugar in Benedict's test for 24
 fermentation test for 24
 quantitative test for 24
 qualitative test for 24
- Urticaria 160
- Uterine haemorrhage treatment of excessive 167
- Uterine tubes 41
- Uterus cyclic changes in 43
 description of 42
- VAGINAL cornification test 133
- Vafotest 196
- Vaso constrictive preparations 193 194
- Vasopressin action and uses of 33 158
 Injection of 158 182 204
 pharmacy of 182
 tannate 182
- Virormone preparations 195
- Vitamin D action of 17
 in tetany 145
- Vitamins and blood sugar 149
- WOMB 42
- Xenopus laevis* in pregnancy test 12 47
 tadpoles in assay test method 129
- ZOOSTEROIDS 62
- Zymosterol 62

